ABSTRACTS COLLECTION

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E-POSTERS

P01 Reproductive Genetics/Prenatal Genetics

P01.001.A Frequency of Y chromosome microdeletions in Turkish infertile men: Single Center Experience

Aysel KalayciYigin, ***Gizem Erdogan****, Deniz Agirbasli, Mehmet Seven*

Department of Medical Genetics, Cerrahpasa Medical Faculty, Istanbul University-Cerrahpasa, Fatih, Turkey.

Objective: Y chromosome microdeletions are the leading genetic cause of male infertility and their detection is clinically relevant for appropriate genetic counseling. Y chromosome includes genes for testicular development and spermatogenesis. The aim of this study was to establish the frequency of the Y chromosome microdeletions in Turkish infertile men who referred to our center with severe oligozoospermia and azoospermia.

Materials and Methods: In our study, 396 infertile men referred to İstanbul University- Cerrahpaşa, Cerrahpaşa Medical Faculty Department of Medical Genetics (GETAM) between 2016 to 2020 with azoospermia/severe oligospermia. We evaluated microdeletions of the Y-chromosome STS markers AZFa, AZFb and AZFc, ZFX/ZFY, terminal sY160 regions by using DNA Fragment analysis.

Results: Among the 396 infertile men, we determined 30 cases of Y chromosome micro- deletions (7.57%). Among 30 cases, AZFc microdeletions were found in 18 cases (60%), AZFa microdeletions in 4 cases (13.3%), AZFb microdeletions in 1 case (3.3%), AZFa,b,c in 4 cases (13.3%), AZFb,c in 3 cases (10%). Our findings are consistent with the literature.

Conclusion: Our results are similar to the previous studies which have mostly reported a frequency of less than 10% for Y chromosome microdeletions. The etiology of infertility remains unknown and novel genes other than y chromosome microdeletions should be identified with high throughput techniques.

A. KalayciYigin: None. **G. Erdogan:** None. **D. Agirbasli:** None. **M. Seven:** None.

P01.002.B Serotonin transporter 5-HTTLPR genotypes and trinucleotide repeats of androgen receptor exert a combinatorial effect on hormonal milieu in patients with lifelong premature ejaculation

Shahzad Bhatti*, Haroon Latif Khan, Sana Abbas, Yousuf Latif Khan*

Lahore Institute of Fertility and Endocrinology, Hameed Latif Hospital, Lahore, Pakistan.

Premature ejaculation is one of the most common sexual disorders in men due to the uncontrolled modulation of spinal reflexes. In this study, we investigate the combinatorial effects of trinucleotide repeats of androgen receptor and allelic variants of the 5-HTTLPR gene on sex steroids, hypophyseal hormones, sexual performance, and premature ejaculation assessment parameters among evidence-based lifelong premature ejaculation subjects. A total of 271 patients consulting for evidence-based lifelong premature ejaculatory dysfunction were selected in this study. The control group consists of 155 men with normal IELT (>4 min). The study revealed that the subjects who have the highest (≥26) CAG stretch depicted significantly higher serum oxytocin levels

(102.1 pg/ml; n = 126, p < 0.001) compared with the control group (71.2 pg/ml; n = 75, p = <0.001). Almost 33 (26.1%) lifelong premature ejaculatory patients had AR variant of longer (≥26) CAG repeats was homozygous for S alleles (SS), 45 (35.7%) was homozygous for L allele (LL), and 48 (38%) had the L/S or S/L genotype of 5-HTTLPR gene. Homozygous (SS) alleles have a significant positive correlation (r = 0.44, p < 0.0001) with the high score of BDI-II (39.1, n = 126, p < 0.001). However, LL alleles have shown a significant positive correlation with PEDT (r = 0.46, p < 0.001) and a negative correlation with self-estimated IELT. The study design elaborates that androgen receptor trinucleotide repeats and 5-HTTLPR genotypes have a combinatorial impact on hormonal milieu and sexual function regarding evidence-based lifelong premature ejaculatory dysfunction patients.

S. Bhatti: None. **H. Latif Khan:** None. **S. Abbas:** None. **Y. Latif Khan:** None.

P01.003.C Challenge in prenatal diagnostics of severe skeletal dysplasias: a case of Achondrogenesis type 2

Natalija Krasovskaja[1,2], *Aušra Matulevičienė*[1,2], *Kamilė Šiaurytė*[1,2], *Gabrielė Žukauskaitė*[2], *Algirdas Utkus*[1]

[1]*Department of Human and Medical Genetics, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, Vilnius, Lithuania,* [2]*Centre for Medical Genetics, Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania.*

Introduction: Achondrogenesis type 2 (ACG2) belongs to a group of most severe type 2 collagen related skeletal dysplasias with autosomal dominant inheritance. Characteristic phenotype features include short stature, extreme micromelia, narrow chest, pulmonary hypoplasia, edema. The condition can be diagnosed prenatally.

Materials and methods: Primegravida, 23 years of age, was referred at 21st week of gestation due to asymmetrical fetal growth restriction. Fetal ultrasound revealed micromelia, narrow chest, prominent abdomen, brachydactyly, talipes, polyhydramnios. Pedigree was uninformative. Sanger sequencing of DNA from amniotic fluid identified no pathogenic changes in the *FGFR3* gene. Next generation sequencing (NGS) of skeletal dysplasia gene panel was performed.

Results: NGS skeletal dysplasia gene panel identified variant NM_001844.5:c.[4387_4389del];[4387=] (rs527236145) in exon 54 of *COL2A1* gene of uncertain clinical significance (*in silico* analysis: Provean: deleterious, Varsome: likely pathogenic). Segregation analysis in the family showed *de novo* origin. Several therapeutic amniocentesis were done to reduce severe polyhydramnios. Pregnancy was carried to 37 gestational weeks. Male newborn was delivered by Caesarean section, birth weight - 2050 g, height - 33 cm, Apgar score - 5/8. Condition quickly derteriorated due to severe pulmonary hypoplasia. Palliative care was administered. Baby died in 11 days.

Conclusions: Precise fetal ultrasound is essential for early suspicion of ACG2. Clinical diagnosis enables advanced diagnostic methods to be applied in timely manner and informed decisions on prenatal and postnatal care to be made.

N. Krasovskaja: None. **A. Matulevičienė:** None. **K. Šiaurytė:** None. **G. Žukauskaitė:** None. **A. Utkus:** None.

P01.004.D The impact of *FMR1 allelic score* in infertile females with preferential X-chromosome inactivation

Bárbara Rodrigues[1,2], *Emídio Vale-Fernandes*[2,3], *Daniela Sousa*[3], *Raquel Brandão*[3], *Nuno Maia*[1,2], *Isabel Marques*[1,2], *Rosário Santos*[1,2], *Carla Leal*[3], *Márcia Barreiro*[3], *António José Arsénia Nogueira*[4], *Paula Jorge*[1,2]

[1]*Molecular Genetics Unit, Centro de Genética Médica Dr. Jacinto Magalhães (CGMJM), Centro Hospitalar Universitário do Porto (CHUP), Porto, Portugal, Porto, Portugal,* [2]*Unit for Multidisciplinary Research in Biomedicine (UMIB), Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, Porto, Portugal, Porto, Portugal,* [3]*Centre for Medically Assisted Procreation/PublicGameteBank, Centro Materno-Infantil do Norte Dr. Albino Aroso (CMIN), Centro Hospitalar Universitário do Porto (CHUP), Porto, Portugal, Porto, Portugal,* [4]*Center for Environmental and Marine Studies (CESAM), Department of Biology, University of Aveiro, Aveiro, Portugal, Aveiro, Portugal.*

Introduction: X-chromosome inactivation (XCI) occurs randomly; however, skewing can occur in 3.2-3.5% of females. The association of XCI skewing with premature ovarian failure and implication of a low *FMR1* gene CGG number (CGGs < 26) in ovarian dysfunction are still controversial. Aiming to test the effect of the AGG interspersions, our group developed a mathematical model that combines the AGG interspersion number and pattern as well as the *FMR1* total repeat length. We then tested if the *FMR1 allelic score* obtained correlates with XCI pattern in females with idiopathic infertility.

Material and Methods: Blood samples from females at reproductive age, in an infertility clinic setting: 40 infertile and 27 potentially fertile females. *Allelic score* of each *FMR1* allele was determined using our mathematical model, as was XCI pattern using HUMARA.

Results: No significant difference was observed between the proportion of infertile cases in each *equivalent* and *dissimilar* allelic combinations, respectively 25/41 and 15/26. In the *equivalent* group one sample carried a 56 CGG premutated allele with two AGG interspersions. The *dissimilar* group was enriched with low *FMR1* CGG genotypes (16/26; 62%), 63% being infertile females as opposed to 39% (16/41) and 50% respectively in the *equivalent* group.

Conclusions: The incidence of highly-skewed XCI (>90:10) was statistically higher in the *dissimilar* group (80% *versus* 50%), suggesting an association between *allelic score* and preferential XCI. Although exploratory, this study suggests that such association may result from a protective *FMR1* AGG interspersion pattern-related effect or unknown X-chromosome-linked anomaly that likely correlates with female infertility.

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P01.006.B How simple is a simple genetic counseling?

Moran Echar[1], *Amir Peleg*[1], *Amalia Harari-Shacham*[1], *Shirley Modan*[1], *Lena Sagi-Dain*[1,2]

[1]*Carmel Medical Center, Haifa, Israel,* [2]*The Ruth and Bruce Rappaport Faculty of Medicine, Technion - Israel Institute of Technology, Haifa, Israel.*

Introduction: Prenatal genetic counseling before amniocentesis in uneventful pregnancies is considered to be a "simple" counseling. In some medical centers the duration of such consultations is limited (to 15 minutes or less), to provide only the basic explanation. The purpose of this study was to evaluate the time required for such supposedly "simple" genetic consultations.

Material and Methods: Data was collected from January 2018 until August 2020 of all patients undergoing genetic counseling before amniocentesis in uneventful pregnancies, and were given by four genetic counselors and two medical geneticists. We have estimated the time required for each consultation.

Results: Of the 1085 consultations, 60.5% required additional explanation. The reasons for extended counseling included medical disorders of the woman or spouse (21.2%), carrier state for autosomal recessive diseases (18.6%), genetic conditions of a child or previous pregnancy (9.6%), or medical disorders in the extended family (79.1%). In 31.0% of patients, carrier screening tests were recommended or added. The additional explanations were estimated as short (up to 5 minutes) in 36.9% of the cases, intermediate (5 to 15 minutes) in 59.9%, and long (over 15 minutes) in 2.6% of cases. The consultation's length was not affected from it being a first or a recurrent consultation.

Discussion: This study reflects the need for a proper genetic consultation for all seemingly simple indications, with an emphasis on detailed personal and family history. As over half of the consultees required extended counseling beyond the basic explanation, assigning sufficient time for the consultation is important.

M. Echar: None. **A. Peleg:** None. **A. Harari-Shacham:** None. **S. Modan:** None. **L. Sagi-Dain:** None.

P01.009.A Genetic analysis of azoospermic men by an integrated NGS panel

Monika Logara Klarić[1], *Lovro Trgovec-Greif*[1], *Lucija Žunić*[2], *Filip Rokić*[1], *Ana Vičić*[3], *Tihana Marić*[4], *Ana Merkler*[5], *Ana Katušić Bojanac*[4], *Robert Belužić*[1], *Feodora Stipoljev*[3], *Oliver Vugrek*[1], *Maja Barbalić*[2,6]

[1]*Ruđer Boškovic Institut, Zagreb, Croatia,* [2]*Genom Ltd., Zagreb, Croatia,* [3]*Department of Obstetrics and Gynecology, Clinical Hospital "Sveti Duh", Zagreb, Croatia,* [4]*Department of Medical Biology, University of Zagreb School of Medicine, Zagreb, Croatia,* [5]*Department of Laboratory Diagnostics, University Hospital Centre Zagreb, Zagreb, Croatia,* [6]*Department of Medical Biology, School of Medicine, University of Split, Split, Croatia.*

Introduction: Y chromosome microdeletions, Klinefelter syndrome and *CFTR* mutations are the leading genetic causes of azoospermia and are all analyzed by different molecular methods. In addition, a number of candidate genes were related to infertility in the last two decades.

Materials/Methods: We designed an NGS amplicon-based panel that simultaneously analyzes all the known above-mentioned genetic variants as well as 11 additional genes recently being associated with azoospermia and ran the analysis on 44 azoospermic men. Twelve samples with known genetic aetiology were used to evaluate the performance of the NGS amplicon-based test. Remaining thirty-two samples consisted of azoospermic men with no defined cause of infertility. The panel consisted of 393 amplicons covering regions of interest. *In house* bioinformatic pipeline was developed to analyse the raw data.

Results: We correctly detected all genetic variants in men with known genetic aetiology. In 32 samples with no defined cause of infertility, we detected three Y chromosome microdeletions and 6 variants in selected genes that passed our filtering criteria for functional impact (in *CFTR*, *SYCE1L*, *TEX15* and *AR)*. Altogether, we detected a genetic cause of azoospermia in 4 individuals and likely causative variants in another 4 out of 32 individuals by running our NGS amplicon-based panel.

Conclusions: This work showed that genetic variants associated with male infertility could be detected by running only one assay. Moreover, customization of the panel with newly discovered genes increases the chance of finding the genetic cause of male patient infertility. Funding: The European Regional Development Fund (KK.01.2.1.01.0113).

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P01.010.B Molecular genetic carrier screening of in the Republic of Sakha (Yakutia) (Russia)

Aitalina Sukhomyasova[1,2], *Anastasia Danilova*[1], *Tatyana Grigorieva*[1,2], *Lutcia Gotovtseva*[1,2], *Polina Golikova*[1], *Nadezhda Maksimova*[1]

[1]*North-Eastern Federal University named after M.K. Ammosov, Medical Institute, Yakutsk, Russian Federation,* [2]*"Republican Hospital No. 1-National Center of Medicine", Yakutsk, Russian Federation.*

Introduction: The Republic of Sakha (Yakutia) is a region of Russia with a high prevalence of some hereditary diseases among the indigenous population, due to genetic and population reasons and the founder effect.With the Medical Genetic Center of the National Center of Medicine for people of Yakut nationality, molecular genetic carrier screening of major mutations with 7 frequent autosomal recessive diseases is carried out: Three M-syndrome, SOPH-syndrome, tyrosinemia type 1, neuronal ceroid lipofuscinosis type 6, non-syndromic type 1 A deafness, type 1 methemoglobinemia, mucopolysaccharidosis-plus syndrome.

Materials and Methods: peripheral blood samples with free informed consent were taken from 404 pregnant women at early prenatal screening and 341 women consulted for pregnancy planning using in vitro fertilization. To detect mutations in 7 diseases all 745 samples were analyzed by real-time PCR.

Results: out of 404 pregnant women examined, 105 (26%) were heterozygous carriers of at least 1 hereditary disease, and 3 pregnant women were carriers of 3 diseases. 3 couples were carriers of the same disease as mucopolysaccharidosis-plus syndrome and SOPH syndrome. They were offered prenatal diagnosis.

Conclusions: a high frequency of mutation carriers is revealed in the Yakut population, which is important both for family planning, as well as for the provision of medical and genetic assistance and the expansion of molecular genetic screening among the population of the Republic of Sakha (Yakutia). The work was carried out within the framework of the state assignment of the Ministry of Science and Higher Education of the Russian Federation (project FSRG-2020-0№14).

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P01.012.D Personalised non-invasive prenatal diagnosis (NIPD) for maternally inherited variants in rare conditions using droplet digital PCR

Joe Shaw[1], ***Ben Paternoster***[1], *Maureen Ramos*[1], *Sarah Nesbitt*[1], *Sophie Sheppard*[1], *Lyn S. Chitty*[1,2], *Natalie Chandler*[1]

[1]*NHS North Thames Genomic Laboratory Hub, London, United Kingdom,* [2]*UCL Great Ormond Street Institute of Child Health, London, United Kingdom.*

Introduction: NIPD for maternally-inherited variants is challenging due to the high background of the maternal variant in cell free DNA. Relative haplotype dosage is used clinically for common X-linked and recessive conditions, but is not suitable for consanguineous couples or those where there is no proband DNA available, and is too expensive to permit validation for rare disorders. Droplet digital PCR (ddPCR) offers the potential for development of NIPD assays personalised to maternal variants, regardless of inheritance type, through relative mutation dosage.

Methods: ddPCR assays were designed for 24 pregnancies at risk of X-linked recessive (14), X-linked dominant (1), autosomal dominant (7) and autosomal recessive (2) conditions. Assays were optimised using maternal genomic DNA, before testing cfDNA extracted from stored maternal plasma obtained from 10 weeks gestation. Fetal fraction was determined using *ZFY* or a paternally inherited SNP for pregnancies bearing male and female fetuses, respectively.

Results: ddPCR testing was concordant with fetal genotype determined following invasive testing in 20 cases. Four cases, all with fetal fractions <4%, were inconclusive. The analysis was modified to reflect the different inheritance patterns, including a case where both parents were affected with the common achondroplasia variant, *FGFR3* c.1138G>A. In this scenario, ddPCR correctly predicted the fetus to be homozygous for the reference allele with a fetal fraction of 2.6%.

Conclusion: ddPCR offers personalised NIPD for maternally inherited variants, using only maternal samples regardless of inheritance pattern. Funding was from the GOSH NIHR Biomedical Research Centre. Samples were obtained from the RAPID sample bank.

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P01.015.C Chromosomal abnormalities in prenataly identified cases with amniocentesis from south of Turkey

Ayfer Pazarbasi[1], *Davut Alptekin*[1], *Sabriye Kocaturk-Sel*[1], *Inayet N. Uslu*[1], *Nermin S. Ilgaz*[1], *Gulsevinc Ay-Aksoy*[1], *Osman Demirhan*[1], *Umit Luleyap*[1], *Mehmet B. Yilmaz*[1], *Selim Buyukkurt*[2]

[1]*Faculty of Medicine, Dept of Medical Biology, Adana, Turkey,* [2]*Faculty of Medicine, Dept of Obstetrics and Gynecology, Adana, Turkey.*

Amniocentesis is a medical procedure used in prenatal diagnosis of chromosomal abnormalities and very crucial for preventing the birth of genetically defective fetuses in order to decrease the prevalence of genetic diseases in populations. A retrospective review of our amniocentesis database for the period from January 2000 to February 2021 was carried out. The karyotyping of 8635 fetuses was carried out in Department of Medical Biology from the samples of amniotic fluids which were sent from Department of Gynecology and Obstetrics of Balcali Hospital. A standart nomenclature has been developed to describe each of types of abnormality found in human chromosomes. A total of 8635 amniocentesis specimens were processed during the study period. 638 fetuses (7.38%) had various chromosomal abnormalities. 54.23% of abnormal karyotypes (346 cases) were numerical and 43.57% (278 cases) were structural. Both numerical and structural chromosomal aberrations were observed in 14 cases (2.19%). The ratios were as: trisomy 21 (49.42%), trisomy 18 (18.49%), monosomy X (9.24%), trisomy 13 (6.64%), Triploidy (4.62%), Klinefelter Syndrome (3.46%), Trisomy X (1. 15%), XYY Syndrome (0.86%), and the others in all numerical abnormalities. The frequent structural abnormalities were as: 46,XX/XY, inv(9) (p11; q12)/(p11;q13)(27.69 %), 46,XX/XY, 1qh(+)(12.58%), 46,XY, Yqh(-) (7.19%), 46,XX/XY, 16qh(+)(6.83%), 46,XX/XY, 9qh(+)(4.31%) and 46,XY, Yqh(+)(4.31%). Balanced and unbalanced translocations, deletions and duplications were also found in less ratio. According to the literature and our results, advanced maternal age is the main cause of fetal chromosomal abnormalities. Fetal chromosomal abnormality ratio that we found was 7.38%. This ratio emphasize the importance of prenatal diagnosis.

A. Pazarbasi: None. **D. Alptekin:** None. **S. Kocaturk-Sel:** None. **I.N. Uslu:** None. **N.S. Ilgaz:** None. **G. Ay-Aksoy:** None. **O. Demirhan:** None. **U. Luleyap:** None. **M.B. Yilmaz:** None. **S. Buyukkurt:** None.

P01.016.D Is it time to report carrier state for recessive disorders in every microarray analysis? - A pilot model based on hearing loss genes deletions

Lena Sagi-Dain[1], ***Idit Maya***[2], *Lina Basel*[2]

[1]*Carmel Medical Center, Haifa, Israel,* [2]*Rabin Medical Center, Petach Tikva, Israel.*

Purpose: To examine the implications of reporting heterozygous losses of recessive genes in Chromosomal Microarray Analysis (CMA), based on the incidence of microdeletions of three common hearing impairment genes in the local cohort and the prevalence of sequence variants in these genes in worldwide databases.

Methods: Prevalence of heterozygous microdeletions in *OTOA* and *STRC* genes, as well as deletions in the DFNB1 locus encompassing *GJB6* gene, was determined using electronic database of Rabin Medical Center. ClinVar archive and Deafness Variation Database were used to generate a list of clinically significant sequence variants in these three genes, as well as *GJB2* gene, and estimation of the frequency of sequence variants was performed.

Results: Of the 19,189 CMA tests were performed in our laboratory, 107 *STRC* microdeletions were found (0.56%), followed in frequency by *OTOA* deletions (39, 0.2%), and DFNB1 locus deletions (10, 0.05%). The estimated risk for a hearing loss in the examined individual carrying the microdeletion was estimated as 0.11-0.67% for *STRC*, 0.016-0.13% for *OTOA*, and 1.9-7.5% in the DFNB1 locus (including double heterozygocity with *GJB2* clinically significant sequence variants). The risks were higher in specific populations.

Conclusions: We believe that that general decision whether to report or to disregard such incidental findings cannot be part of a uniform policy, but rather based on a detailed evaluation of origin-specific variants for each gene, with a careful consideration and discussion whether to include the microdeletion in the final report for each patient.

L. Sagi-Dain: None. **I. Maya:** None. **L. Basel:** None.

P01.018.B Prenatal craniofacial malformations should be analysed by whole exome sequencing in addition to chromosomal microarray analysis

Rachel Michaelson-Cohen[1], *Amihood Singer*[2], *Lena Sagi-Dain*[3], *Reeval Segel*[1]

[1]*Shaare Zedek Medical Center, Hebrew University, Jerusalem Israel, Jerusalem, Israel,* [2]*Department of Community Genetics, Public Health Services, Ministry of Health, Jerusalem, Israel,* [3]*Genetics Institute, Carmel Medical Center, Rappaport Faculty of Medicine, Technion Institute of Technology, Haifa, Israel.*

Introduction: Craniofacial malformations (CFMs) account for approximately 15% of congenital malformations. Genetic evaluation is important for decision-making processes in couples dealing with fetal CFMs, yet previous assessments are limited. Our objective was to examine the detection rate of clinically significant chromosomal microarray analysis (CMA) findings in pregnancies with CFMs.

Methods: Data from all CMA tests in pregnancies with sonographic diagnosis of CFMs (cleft lip and/or palate, malformations of eyes, nose or ears, micro-retrognathia etc) performed between January 2016 and April 2020 were retrospectively obtained from the Israeli Ministry of Health computerized database. Rates of clinically significant CMA results in fetuses with CFMs were compared to baseline risk, based on a local cohort of pregnancies with no major sonographic anomalies.

Results: A total of 111 CMA tests were performed due to fetal CFMs. In the 18 pregnancies with non-isolated CFMs, three (16.7%) clinically significant CMA results were detected, a significantly higher frequency compared to the control cohort, for whom the rate of pathogenic CMA is 1.4% (RR 14.1 (95% CI 3.7-54.2)). Of the 93 cases with isolated CFMs, four (4.3%) clinically significant pathogenic CMA results were detected, a rate slightly increased compared to the control population (RR 3.17 (95% CI 1.02-9.83)).

Discussion: Fetal CFMs diagnosed by sonogram, whether isolated or associated with additional sonographic defects, are associated with abnormal CMA findings. However, when isolated, abnormal CMA rate is only slightly higher than the background risk. Therefore, combining CMA and whole exome sequencing should be considered for optimizing genetic evaluation of CFMs.

R. Michaelson-Cohen: None. **A. Singer:** None. **L. Sagi-Dain:** None. **R. Segel:** None.

P01.019.C Web-based interactive educational tool to prepare couples for prenatal chromosomal-microarray-analysis (CMA)

*Hagit Hochner[1], Talya Millo[2], Gil Siegal[3], **Shiri Shkedi-Rafid**[4]*

[1]Braun School of Public Health, the Hebrew University of Jerusalem, Jerusalem, Israel, [2]Hebrew University, Jerusalem, Israel, [3]Ono Academic Center, Center for Health Law, Bioethics and Health Policy, Kiryat Ono, Israel, [4]Hadassah Hebrew University Medical Center, Jerusalem, Israel.

Introduction: Results from prenatal Chromosomal-microarray-analysis (CMA) include variants with uncertain clinical significance (VUS), low-penetrance susceptibility-loci (SL) and risks for late-onset conditions. Some medical centers offer parents the choice if to be informed about these findings. We set-out to design and implement a web-based interactive educational tool for women/couples undergoing prenatal CMA aimed to assist in making informed decisions and improve their preparation for potential findings.

Methods: Development of the tool was based on interviews with women/their partners (N = 42) following prenatal CMA to explore their experience with parental choice. Knowledge and feedback questions were incorporated into the web-based tool, that was offered to women/couples prior to the CMA-test. Uptake, knowledge and satisfaction were evaluated on the first 180 users.

Results: About 80% of the women who logged into the system completed the process. Distribution of choices made using the educational tool was 88.9%, 63.6%, and 57.6% for disclosure of late-onset, SL and VUS findings, respectively, similar to distribution of choices published prior the tool's implementation. The majority of respondents answered the knowledge questions correctly (range 88.8% to 98.7%). Reported satisfaction was highest for the use of animated videos (85.1%), 76.0% of the respondents felt they were better prepared for CMA testing and 78.1% indicated that they would recommend the tool to others. Decision-aid based on interviews data was reported helpful by 63.4% of respondents.

Conclusions: A pre-CMA test interactive web-based educational tool is well received and valued by women/couples and assists in making informed decisions regarding the disclosure of complex genomic-results.

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P01.020.D Prenatal findings of cataract and arthrogryposis: recurrence of cerebro-oculo-facio-skeletal syndrome and review of differential diagnosis

***Fabio Sirchia**[1], Ilaria Fantasia[2], Agnese Feresin[3], Elisa Giorgio[1], Moira Barbieri[3], Valentina Guida[4], Alessandro De Luca[4], Tamara Stampalija[3,2]*

[1]University of Pavia, Pavia, Italy, [2]Burlo Garofolo Hospital, Trieste, Italy, [3]University of Trieste, Trieste, Italy, [4]Division of Medical Genetics, Fondazione IRCCS-Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy.

Cerebro-oculo-facio-skeletal syndrome (COFS) is a severe and progressive neurologic condition characterized by prenatal onset of arthrogryposis, cataract, microcephaly and growth failure. The aim of this study was to present a case of recurrence of the COFS syndrome and to propose a differential diagnosis flow-chart in case of prenatal findings of arthrogryposis and cataract.

We report a case of recurrence of COFS3 syndrome within the same family, with similar diagnostic features. In the first case the COFS syndrome remainedundiagnosed, while in the second case, due to prenatal findings of arthrogryposis and cataract, genetic investigation focusing on responsible genes of COFS (ERCC5, ERCC6 and FKTN genes) was carried out. The fetus was found to be compound heterozygous for two different ERCC5 mutations, confirming the clinical suspect of COFS syndrome. A review of the literature on possible causative genes of prenatal cataract and arthrogryposis was performed and we present a flow-chart to guide differential diagnosis and possible genetic testing in case of these findings.

COFS syndrome is a rare autosomic recessive condition. However, it can besuspected and diagnosed prenatally. The flow-chart illustrates a pathway to guide differential diagnosis according to the prenatal findings. Main syndromes, key testing and specific genes are included. Targeted molecular testing should be offered to the couple in order to reach a diagnosis and assess the recurrence risk for future pregnancies.

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P01.021.A Hypomorphic variants in FLNA cause isolated congenital anomalies of kidney and urinary tract in a large family

***Shalini S. Nayak**, Shravya MS, Katta M. Girisha*

Kasturba Medical College, Manipal, Manipal, India.

Introduction: *FLNA* encodes filamin A, an actin binding protein that regulates the reorganization of the actin cytoskeleton by interacting with integrins, transmembrane receptor complexes

and secondary messengers. *FLNA* is known to cause several X-linked allelic diseases. Recently unrelated four individuals were noted to have isolated congenital anomalies of the kidney and urinary tract (CAKUT) and hypomorphic variants in *FLNA*. We hereby report a family with six affected offspring's with CACUT.

Methods: We evaluated a consanguineous family with three-years-old male living child and five abortions. We performed chromosomal microarray followed by duo exome sequencing for the living proband and sixth abortuses.

Results: The family had bilateral renal pyelectasis in first pregnancy, gross ascites in second conceptus and cardiac anomalies in fifth pregnancy. However, a detailed postnatal examination was not possible. Autopsy from third pregnancy revealed bladder outlet obstruction whereas the sixth pregnancy had bilateral tortuous ureters. The living child has hydroureteronephrosis, mildly impaired functioning of left kidney and preserved functioning of right kidney with non-obstructive clearance. Exome sequencing identified a novel hemizygous variant, c.7282G>A; p. (Gly2428Arg) in exon 44 of *FLNA*, mother is a carrier and father has wild type allele.

Conclusion: The hypomorphic variant c.7282G>A in *FLNA* is the likely genetic cause responsible for CAKUT seen in this family. However, the segregation analysis in other affected fetuses was not possible due to unavailability of DNA. Also, there was limited phenotypic data in fifth pregnancy to explain the cardiac anomaly.

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P01.022.B Two prenatal cases with a variant of loss in homozygosity involving the *CRPPA* (*ISPD*) gene

Cíntia Ventura[1], *Raquel Lemos*[1], *Patríca Costa*[1], *Marisa Teixeira*[1], *Joaquim Sá*[1], *Gabriela Soares*[1], *Martin Mistrík*[2], *Rita Cerqueira*[1]

[1]*CGC Genetics UNILABS Portugal, Porto, Portugal,* [2]*Alpha Medical UNILABS Eslováquia, Zilinsky, Slovakia.*

Introduction: Homozygous mutations of the *CRPPA* gene [MIM 614631] are associated with congenital muscular dystrophy-dystroglycanopathy with brain and eye anomalies (type A7) described by OMIM [MIM 614643] including features such as hydrocephalus and being the cause of the most severe phenotype of Walker-Warburg syndrome. We present, in the context of prenatal diagnosis, two cases with aCGH results involving a homozygous deletion that includes the *CRPPA* gene. The fetus in case 1 presented hydrocephalus and abnormal morphology - Dandy Walker malformation. The fetus in case 2 presented hydrocephalus.

Methodology: The aCGH was performed using Affymetrix Cytoscan 750K. PCR amplification of exons 1 and 9 of the *CRPPA* gene (chromosome 7) was performed.

Results: Case1 - A female genomic profile was detected with a loss in homozygosity (zero copies) in 7p21.2p21.1 of 360 Kbp involving the *CRPPA, CRPPA-AS1 and SOSTDC1* genes. No amplification of exons 1 and 9 of the *CRPPA* gene was observed, confirming the findings of aCGH. Case 2 - A female genomic profile was detected with a loss in homozygosity (zero copies) in 7p21.2p21.1 of 360 Kbp involving the *CRPPA, CRPPA-AS1 and SOSTDC1* genes. These variants are classified as pathogenic and may correspond to these fetuses' phenotypes.

Conclusions: These findings reinforce the importance of aCGH as a first-line diagnostic test in fetuses with ultrasound anomalies. Genetic Counseling is imperative to guide the couple in the best decision as well as to warn of the need for further analysis in the family.

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P01.023.C Three foetuses with Cornelia de Lange diagnosis: prenatal findings and genetic diagnosis

Fe Amalia García Santiago[1,2], *Elena Mansilla*[1,2], *Eugenia Antolin*[3], *Fernando Santos Simarro*[1,2], *Roberto Rodriguez*[3], *Julian Nevado*[4,2], *Maria Palomares-Bralo*[4,2], *Karen Heath*[1,2], *Rita Maria Regojo*[5], *Carmen Rodriguez-Jimenez*[6], *Isabel Vallcorba*[6], *Angela del Pozo*[4], *Pablo Lapunzina*[4,2]

[1]*INGEMM, Hospital Universitario La Paz, Idipaz, UMDE, Ciberer, Madrid, Spain,* [2]*Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER, U753), Instituto Carlos III, Madrid, Spain., Madrid, Spain,* [3]*Department of Obstetrics and Gynaecology, Hospital Universitario La Paz, Idipaz, UMDE, Madrid, Spain,* [4]*INGEMM, Hospital Universitario La Paz, Idipaz, Ciberer, Madrid, Spain,* [5]*Department of Pathology, Hospital Universitario La Paz, UMDE, Madrid, Spain,* [6]*INGEMM, Hospital Universitario La Paz, Madrid, Spain.*

Introduction: Cornelia de Lange syndrome (CdLS; MIM #12270, 300590, 610759, 614701, 300882) is a rare and clinically variable disorder that affects multiple organs. Ultrasound findings are highly variable and include distinctive facial features, prenatal growth retardation (FGR), malformations of the upper limbs, diaphragmatic hernia, heart defects and genitourinary malformations. To date, five genes (NIPBL, SMC1A, SMC3, RAD21 and HDAC8) have been associated with CdLS. We report 3 foetuses with ultrasound characteristics and pathology examination with abnormalities associated with CdLS.

Materials and Methods: an NGS custom panel containing 1663 genes involved in common genetic disorders (RD seq (R) v6.0), exome trio and MLPA (multiplex-ligation dependent probe amplification) P141 (MRC-Holland) were performed after a CMA normal result.

Results: foetus 1 (23 weeks): severe FGR, ulnar hypoplasia and oligodactyly of right hand, hypospadias, left diaphragmatic hernia, prefrontal edema, retrognathia. Genetic analysis: a loss of dosage in heterozygosity is detected in the probe hybridising to exon 43 of the *NIPBL* gene by MLPA thecnique. Foetus 2: (21 weeks): severe FGR, micromelia, congenital heart, prefrontal edema, microcephalia, short corpus callosum. Genetic analysis: *NIPBL* NM_133433.3: c.5639_5642del, p.Pro1880Hisfs*10 heterocygosis. Foetus 3: (17 weeks): severe FRG, radius hypoplasia and oligodactyly of left hand, left diaphragmatic hernia, micrognathia. Genetic analysis: *NIPBL* NM_133433.3:c.598C>T;p./Gln200*) heterocygosis.

Conclusions: CdLS is a heterogeneous clinical and genetic condition. It is essential to have a thorough ultrasound examination and to perform this genetic study in cases of FGR that cannot be explained by chromosomal or vascular alterations.

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P01.024.D Transcriptome landscape of the human decidual cells

Anastasia A. Babovskaya, *Ekaterina Trifonova, Maria Swarovskaya, Viktoria Serebrova, Alexei Zarubin, Vadim Stepanov*

Research Institute of Medical Genetics, Tomsk National Research Medical Center of the Russian Academy of Sciences, Tomsk, Russian Federation.

Human reproductive success depends on a properly decidualized uterine endometrium that allows implantation and the formation of the placenta. At the core of the decidualization process are endometrial fibroblasts that differentiate to decidual stromal cells (DSCs). Characterizing transcriptome of DSC, which is crucial for a pregnancy's outcome, can serve as a basis for identifying the mechanisms underlying physiological and pathological pregnancy. In our study for the first time for native cells high-throughput sequencing (RNA-seq) was applied to analyze the global transcriptome of the human DSCs during uncomplicated pregnancies. DSCs were obtained by Laser capture microdissection. During analyses we obtained 17960 transcripts expressed with CPM >1 in each sample. Most of the analyzed transcripts corresponded to protein coding regions of the human genome (13683). Antisense RNAs, long noncoding RNAs, and processed pseudogenes predominated in the remaining cluster. To gain a better understanding of the biological implications, the assembled transcripts were annotated using DAVID Bioinformatics Resources. The top 5 represented GO terms for the biological process were cell-cell adhesion, transcription, protein transport, rRNA processing and proteasome-mediated ubiquitin-dependent protein catabolic process. The findings suggest that human DSCs play a key role in the induction of maternal-fetal communication. We applied an upstream analysis approach implemented in geneXplain platform and identified top 10 master regulators (DUSP10, MOS, ROCK2, MAPK6, HTT, SGK1, PRKCI, PASK, DUSP22, and CUX1). These key genes may be potential biomarkers of diagnosis or new therapeutic targets for pregnancy complications.The reported study was funded by RFBR №20-34-90128, №18-29-13045.

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P01.025.A Novel pathogenic c.34G>C mutation in *GATA4* gene detected in 46,XY DSD patient from Ukraine. The evidence for autosomal dominant DSD inheritance with incomplete penetrance in women

Ludmila Livshits[1], *Dmytro Sirokha*[1], *Olexandra Gorodna*[1], *Kamila Kusz-Zamelczyk*[2], *Patrick Sproll*[3], *Anna Lauber Biason*[3], *Serge Nef*[4], *Nataliya Zelinska*[5]

[1]*Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, Kyiv, Ukraine,* [2]*Institute of Human Genetics, Polish Academy of Sciences, Poznań, Poland,* [3]*Section of Medicine, University of Fribourg, Fribourg, Switzerland,* [4]*Faculty of Medicine, University of Geneva, Geneva, Switzerland,* [5]*Ukrainian Scientific and Practical Center for Endocrine Surgery, Transplantation of Endocrine Organs and Tissues, Ministry of Health of Ukraine, Kyiv, Ukraine.*

Disorders of sexual development (DSD) are an important group of rare human diseases. To date there are more than 150 known genes involved in DSD and up to 1000 candidates possibly implicated in gonadal development. The aim of the research was to identify novel DSD genetic variants using whole exome sequencing (WES). The WES was performed for a 46,XY SRY positive patient with gonadal dysgenesis. A c.34G>C (rs750597721) mutation in *GATA4* gene was identified and confirmed as pathogenic using bioinformatic tools. This is a heterozygous missense substitution that leads to Gly12Ala mutation in a protein sequence that corresponds to transactivation domain 1. Sanger sequencing analysis conducted in family members revealed that healthy mother and healthy maternal grandmother are heterozygous carriers of c.34G>C mutation as well. Previously, another heterozygous mutations in *GATA4* were detected in 46,XY DSD patients. Interestingly, the same mutation was previously shown in patient with Atrioventricular septal defect 4. Obtained data are the evidence of phenotypic variation and incomplete penetrance in women. It is in line with previously obtained explanation for possible molecular mechanisms of such inheritance in mice (Bouma et. al., 2007). To our knowledge, this is the first report on c.34G>C in patient with 46,XY DSD and the results of family member analysis support the hypothesis that this variant is causative for 46,XY DSD with autosomal dominant inheritance and incomplete penetrance in women. Study was performed as a part of SCOPES 2013-2017: Joint Research Projects - Genetics of Human Disorders of Sexual Development.

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P01.026.B Prenatal detection of a familial 640 kb microdeletion in chromosomal region 6q27 in a fetus with isolated severe bilateral ventriculomegaly

Yvonne Stratis[1], *Cornelie Müller-Hofstede*[1], *Malvina Kuschmann*[1], *Matthias Meyer-Wittkopf*[2], *Axel Bohring*[1], *Albrecht Röpke*[1]

[1]*Institute of Human Genetics, Münster, Germany,* [2]*Department of Gynecology and Obstetrics at the Health Center Rheine, Mathias Spital, Rheine, Germany.*

The phenotype associated with terminal deletions of the chromosome 6q27 region includes intellectual disability, seizures and multiple brain malformations. The most common brain malformations are corpus callosum abnormalities, periventricular nodular heterotopia, polymicrogyria, hydrocephalus, ventriculomegaly and cerebellar malformations. The smallest region of overlap for the phenotype of brain malformations and intellectual disability is refined to a segment of 325 kb in 6q27, comprising the protein coding genes *DLL1*, *PSMB1*, *TBP* and *PDCD2*. Haploinsufficiency of DLL1, which is a NOTCH ligand, causes a neurodevelopmental disorder with nonspecific brain abnormalities with or without seizures. *TBP* is a candidate gene for ID and is linked to PDCD2 and PSMB1 in a conserved manner, suggesting a potential interaction between these genes. Here we report on an inherited 640 kb terminal 6q27 deletion in a 29-week fetus with isolated severe bilateral ventriculomegaly detected by SNP-array on uncultured amniocytes. The deletion comprises six genes, including the aforementioned protein coding genes *DLL1*, *PSMB1*, *TBP* and *PDCD2* which define the minimal critical region. FISH-analysis of cultured amniocytes confirmed the deletion on metaphase cells. SNP-array and FISH analysis showed that the mildly affected mother harbors the same 6q27 deletion. Prenatal diagnosis of 6q27 deletion is extremely rare and to the best of our knowledge only nine patients have been reported yet. Of these, only four cases had isolated ventriculomegaly, whereas five cases had additional malformations. Our case clearly demonstrates the importance of performing prenatal array analysis also in cases of isolated bilateral ventriculomegaly.

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P01.027.C Telomere length in individuals with early pregnancy losses

Nataliya Huleyuk[1], *Danuta Zastavna*[1], *Iryna Tkach*[1], *Ivanna Haiboniuk*[1], *Miroslaw Tyrka*[2]

[1]*Institute of Hereditary Pathology of AMS Ukraine, Lviv, Ukraine,* [2]*Department of Biotechnology and Bioinformatics, Faculty of Chemistry, Rzeszow University of Technology, Rzeszow, Poland.*

Introduction: Over the past decade, telomere biology has become an important topic in the field of human reproduction. Early pregnancy loss (EPL) occurs in ~ 15% of clinically-recognized pregnancies and is the most common complication of pregnancy. Spontaneously lost pregnancies are characterized by shortened telomeres. We focused on the relationship between relative telomere length (RTL) and tendency to EPL in humans.

Material and Methods: Relative telomere length was measured in DNA isolated from the blood samples using a real-time polymerase chain reaction approach. RTL was examined in control group (C) (N = 209) - women (CW) (N = 107) and men (CM) (N = 102) who had healthy pregnancies with no history of infertility or miscarriage, and in group with EPL (N = 445) - women (EPLW) (N = 223) and men (EPLM) (N = 212) who had single or more EPL. RTL data were analysed by gender and reproductive history.

Results: Women (CW+EPLW) have significantly higher RTL that men (CM+EPLM) (1.74 ± 0.06 in women and 1.40 ± 0.05 in men, P = 0.000053). Average RTL were significantly lower in CM compared to CW (CW:2.27 ± 0.12 versus CM: 1.15 ± 0.08, P = 0.0000001), and were similar in EPLW and EPLM (1.50 ± 0.06 in EPLW and 1.53 ± 0.06 in EPLM, P = 0.47). The EPLW group had significantly lower RTL than control (EPLW:1.50 ± 0.06 versus CW:2.27 ± 0.12, P = 0.0000001). Average RTL were significantly lower in CM compared to EPLM (1.15 ± 0.08 in CM and 1.53 ± 0.06 in EPLM, P = 0.00006).

Conclusions: Women with no history of EPL have longer telomere that men. Woman with EPL have shorter telomere that women without miscarriage. In EPL group women and men have similar telomere length.

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P01.028.D Genetic basis of endometriosis in the susceptibility of developing gynecological cancers

Aintzane Rueda-Martínez[1,2], *Bárbara Paola González-García*[1,2], *Aiara Garitazelaia*[1,2], *Ariadna Cilleros-Portet*[1,2], *Sergi Marí*[1,2], *Rebeca Arauzo*[1,2], *Jokin de Miguel*[1,2], *Nora Fernandez-Jimenez*[1,2], *Jose Ramon Bilbao*[1,2,3], ***Iraia García-Santisteban***[1,2]

[1]*University of the Basque Country (UPV/EHU), Leioa, Spain,* [2]*Biocruces-Bizkaia Health Research Institute, Barakaldo, Spain,* [3]*Spanish Biomedical Research Center in Diabetes and associated Metabolic Disorders, Madrid, Spain.*

Introduction: Endometriosis is a common gynecological disorder in which the endometrium grows outside of the uterus. Despite being considered a benign condition, epidemiological evidence shows that women with endometriosis develop more frequently certain types of gynecological cancers, including endometrial, breast and ovarian carcinomas. However, the mechanisms underlying this relationship remain uncertain. Since genetic factors play a key role in these complex gynecological diseases, we hypothesized that the biological mechanisms behind this comorbidity could be mediated, at least in part, by shared genetic predisposition factors.

Materials and Methods: To test this hypothesis, we undertook a Bioinformatics approach that consisted of a cross-disorder meta-analysis and a Two-Sample Mendelian Randomization (2SMR) analysis of results from public GWASs on endometriosis and the abovementioned gynecological cancers.

Results: Firstly, our meta-analysis revealed novel susceptibility *loci* shared between endometriosis and endometrial, breast, and ovarian cancers, although the input of endometriosis was minor when compared to the cancer studies. Secondly, our 2SMR analysis confirmed previously reported genetic pleiotropy between endometriosis and endometrial cancer but gave inconclusive results about breast cancer, also in line with previous reports. Our research provides, for the first time, solid evidence of a causal genetic association between endometriosis and ovarian cancer, particularly clear cell type, and endometroid subtypes. Furthermore, we also identified genetic variants that could mediate in those associations, allowing future functional experiments.

Conclusions: This study represents the first Bioinformatics approach to elucidate the causal genetic relationship between endometriosis and gynecological cancers. Funding: GVSAN2020/111043, GVSAN2018/111086, and GVSAN2019/111085 to I.G.-S., J. R.B., and N.F.-J., respectively.

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P01.029.A Assisted reproductive technology can be a risk for epimutation-mediated imprinting disorders for mothers over 30 years

Kaori Hara-Isono[1], *Keiko Matsubara*[1], *Masashi Mikami*[2], *Takahiro Arima*[3], *Tsutomu Ogata*[4], *Maki Fukami*[1], *Masayo Kagami*[1]

[1]*National Research Institute for Child Health and Development, Tokyo, Japan,* [2]*National Center for Child Health and Development, Tokyo, Japan,* [3]*Tohoku University Graduate School of Medicine, Sendai, Japan,* [4]*Hamamatsu University School of Medicine, Hamamatsu, Japan.*

Backgrounds: The proportion of assisted reproductive technology (ART)-conceived livebirths of patients with imprinting disorders (IDs) is higher than that of the general population. Whether this is due to ART or confounding effects of advanced parental age was unknown. The aims of this study are 1) to clarify whether ART or maternal ages facilitates development of epimutation-mediated IDs (epi-IDs), and 2) to identify the differentially methylated region (DMR) that is vulnerable to the effect of ART and parental ages.

Results: We enrolled 136 patients with epi-IDs and obtained general population ART data from the Japanese robust nationwide registry. We compared the proportion of ART-conceived livebirths and maternal childbearing ages between patients with epi-IDs and the general population. The proportion of ART-conceived livebirths in patients with epi-IDs was higher than that in mothers aged ≥ 30 years, the age group in which more than 90% of ART procedures performed. The maternal childbearing ages of patients with epi-IDs were widely distributed from 19 to 45 (median: 32). In addition, we compared the proportion of ART-conceived livebirths and parental ages at childbirth across patients with eight epi-IDs. We demonstrated that most ART-conceived patients with epi-IDs were found in Silver-Russell syndrome (SRS) and Beckwith-Wiedemann syndrome (BWS) patients, and parental ages were almost consistent in patients with eight epi-IDs.

Conclusions: ART can be a risk factor for the development of epi-IDs for mothers aged ≥ 30 years. In addition, methylation status of SRS- and BWS- related DMRs may be vulnerable to the effects of ART.

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P01.030.B Analyzing the effects of *ETV5* and *CXCL12* genes in patients with Sertoli cell-only syndrome

O. Sena AYDOS[1], *Dunya AYDOS*[2], *Yunus YUKSELTEN*[3], *Asuman SUNGUROGLU*[1], *Kaan AYDOS*[4]

[1]*Department of Medical Biology, Ankara University School of Medicine, Ankara, Turkey,* [2]*Ankara University Stem Cell Institute, Ankara, Turkey,* [3]*Research Laboratories for Health Science, Y Gen Biotechnology Company Ltd., Ankara, Turkey,* [4]*Department of Urology, Ankara University School of Medicine, Ankara, Turkey.*

Introduction: Ets variant gene 5 (*ETV5*), belongs to a family of transcription factors, regulates several genes essential for spermatogonial stem cells (SSCs) self-renewal. Silencing of *ETV5* in mice led to total loss of stem/progenitor spermatogonia, resulting in Sertoli cell-only (SCO) phenotype. ETV5-deficient Sertoli cells were also found to have decreased CXCL12 levels. In embryonic mouse gonads, CXCL12 was shown to direct the migration of primordial germ cells (PGCs) to the gonadal ridges. We aimed to investigate the role of interaction between *ETV5* and *CXCL12* genes in humans with SCO syndrome.

Materials and methods: Fold changes in expression levels of *CXCL12* and *ETV5* were determined by quantitative PCR in non-obstructive azoospermia (NOA) ($n = 10$) patients with SCOS and obstructive azoospermia (OA) ($n = 2$) as control cases. Testicular tissue samples were taken during microTESE attempt and testicular biopsy was performed for histopathological evaluation.

Results: Fold decreases in *CXCL12* and *ETV5* expression levels were found as 0.44 ± 0.1 and 0.27 ± 0.11, respectively, and the differences were significant compared to controls ($p < 0.001$).

Conclusions: According to our results, the decrease in *CXCL12* and *ETV5* expression levels in patients with SCOS indicated that *CXCL12* and *ETV5* work together in harmony to regulate the presence and maintenance of SSCs in humans. Whether the germ cell loss is due to inhibition of PGCs migration or impaired differentiation of SSCs needs further investigation.

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P01.031.C Exome sequencing in structurally normal fetuses - yield and dilemmas

Hagit Daum, *Tamar Harel, Shiri Gershon-Naamat, Adily Basal, Orly Elpeleg, Vardiella Meiner, Hagar Mor-Shaked*

Hadassah, Jerusalem, Israel.

Abstract Introduction: Although there is a growing body of literature regarding the yield of chromosomal microarray analysis for structurally normal fetuses, there is no data regarding the yield of exome sequencing in this population. Exome sequencing (ES) is a powerful tool for identifying disease-causing single nucleotide variants (SNVs) and small indels. In the prenatal setup, when the clinical phenotyping of the fetus is frequently vague and non-specific, the contribution of exome sequencing to the diagnostic procedure is invaluable.

Material and methods: From February 2017 to February 2021, a total of 635 fetal exomes were analyzed at our center. Among these were 119 structurally normal fetuses. Analysis included SNVs, copy number variants and uniparental disomy. Reports included pathogenic and likely pathogenic findings, including ACMG secondary findings that are relevant during childhood.

Results: Two fetuses (1.7%) had molecular diagnoses of moderate to severe disease severity. Pathogenic compound heterozygous variants were identified in *ATP7B* gene and *NR2E3*, responsible for Wilson disease and for Enhanced S-cone syndrome, respectively. Notably, Wilson disease is a potentially treatable disease and early diagnosis is critical to postnatal management.

Conclusions: With careful management and restrictive analysis, prenatal ES should be considered as an adjunct to chromosomal microarray in sonographically normal fetuses. Further studies are called for in order to determine the yield of prenatal ES in this setting.

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P01.032.D Genetic variants of MTHFR and TNF-α in fetal growth restriction

dema alset[1], *Elena Butenko*[1], *Ekaterina Zabanova*[2], *Natalia Kuznetsova*[2]

[1]*southern federal university, Rostov-on-Don, Russian Federation,* [2]*Rostov State Medical University, Rostov-on-Don, Russian Federation.*

Introduction: Fetal Growth Restriction (FGR) is a multifactorial condition in which fetus cannot reach its genetically determined potential size. This syndrome is considered as an important cause of fetal morbidity and mortality and affects 5-10% of all pregnancies worldwide and 5-17.6% in Russia. Clinician trials are concerning maternal, fetal and placental polymorphisms as possible prenatal FGR-markers. This study aimed to investigate associations of two polymorphisms: 5,10–Methylenetetrahydrofolate reductase/ MTHFR(677T) and Tumor necrosis factor alpha/TNF-α(G308A) with FGR risk.

Material and methods: Using PRISMA statement, 20 studies were included in meta-analysis of MTHFR (C677T) and TNF-α (G308A) associations with FGR. Then MTHFR (C677T) genotyping was conducted with Allele-specific PCR to confirm meta-analysis result in FGR-diagnosed ($n = 26$) and healthy ($n = 37$) pregnant women.

Results: Meta-analysis showed no association of TNF-α(G308A) but a strong association of MTHFR(C677T) with high FGR risk (OR = 1.22, 95 % CI: 1.07-1.39, P = 0.002). However, Genotyping showed that in the studied population, MTHFR(C677T) has no significant association with FGR (OR = 0.917, 95 % CI: 0.328-2.560, P = 0.956).

Conclusion: To our knowledge, this is the first meta-analysis of TNF-α(G308A) in FGR. About MTHFR(C677T), genotyping result was inconsistent with meta-analysis. This suggests that association differs according to ethnicity, since some of the studies included in meta-analysis figured no association in their samples. However, due to our small sample size, further clinical trials are required to confirm results in the studied population.This study was funded by the Ministry of Science and Higher Education of the Russian Federation #0852-2020-0028.

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P01.033.A The FTO gene and adolescent puberty

Elena Mashkina, *Maria Amelina, Michail Shkurat*

Southern Federal University, Rostov-on-Don, Russian Federation.

Introduction: The time of the onset of puberty and obesity are multifactorial features of human. The aim of this work was to analyze the association between the FTO 23525T>A gene polymorphism and the delayed sexual development in boys aged 11-15 years.

Material and methods: DNA samples isolated from blood cells of 322 adolescents were used. The boys were divided into 2 groups - with a normal rate of sexual development and with a delay in sexual development. The stages of sexual development were determined using the Tanner scale. Allele-specific PCR was used to analyze the rs99305069 FTO.

Results: We analyzed the frequencies of genotypes for the FTO gene 23525T>A polymorphism among 11-15 year old boys depending on the stage of puberty. Heterozygotes 23525TA of the FTO gene predominated (45%) among boys with normal sexual development. The frequency of homozygotes for the 23525A allele was 28% in this group. In the group with delay sexual development the frequency of 23525AA genotype was 40%. Homozygotes 23525AA have an increased risk of delayed sexual development during puberty (OR = 1.38 CI 1.1-2.78). Also the 23525A allele of the FTO gene is more frequently recorded among adolescents with delay in sexual development ($\chi 2 = 6.26$ $p = 0.01$; OR = 1.49 (1.09-2.04)). Thus, an association of the 23525T>A polymorphism of the FTO gene with a disturbance in the rate of puberty in adolescents was revealed. This study was funded by the Ministry of Science and Higher Education of the Russian Federation #0852-2020-0028.

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P01.034.B Genetic counseling and carrier screening of candidates for gamete donation at a public bank

Celia Azevedo Soares[1], *Ana R. Soares*[1], *Emídio Vale Fernandes*[2], *Maria Abreu*[1], *Cláudia Falcão Reis*[1], *Ana M. Fortuna*[1], *Natália Tkachenko*[1]

[1]*Centro de Genética Médica Jacinto Magalhães, Porto, Portugal,* [2]*Centro Materno Infantil do Norte, Porto, Portugal.*

Introduction: Genetic counseling and carrier screening of healthy candidates is part of gamete donors' selection. We aim to review the findings of the genetic counseling of a cohort of patients at our public gametes bank.

Methods: Thirty-four male and 64 female candidates had genetic counseling with a medical geneticist before donation. Of these, one female candidate voluntarily dropped-out. Thirty-four males and 63 females performed karyotype and screening for the more common pathogenic variants of *CFTR*-related cystic fibrosis and spinal muscular atrophy (*SMN1*) in the Portuguese population. In addition, all females also performed Fragile X expansion screening (*FMR1*). Thirty patients with ancestry from Southern or Central Portugal, or with known or assumed African ancestry performed hemoglobinopathies screening.

Results: Six patients were withheld from the donation process given their family or personal history that required further investigation. Of the initial 97 candidates, 15.5% presented anomalous laboratory results (15/97). Ten patients were carriers for an autosomal recessive disorder - cystic fibrosis (5/97), sickle cell anemia (3/30), and spinal muscular atrophy (2/97). One female was an *FMR1* pre-mutation carrier (1/63). One female patient presented with triple X mosaicism: 47,XXX[2]/46,XX[50]. Two patients presented with chromosomal instability of unknown origin. In one patient, a mosaic for the Philadelphia chromosome was detected, revealing the unexpected diagnosis of chronic myeloid leukemia.

Conclusions: From a cohort of 97 candidates, 21.7% presented a family/personal history or an anomalous laboratory result that required additional genetic counseling, stressing the importance of performing pre-donation genetic counseling in this population.

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P01.035.C Ascertainment of genome-wide androgenetic mosaicism after discordant results from primary fetal samples and cultured cells

Gioia Mastromoro[1], *Daniele Guadagnolo*[1], *Enrica Marchionni*[1], *Francesca Di Palma*[1], *Barbara Torres*[2], *Marina Goldoni*[2], *Annamaria Onori*[2], *Laura Bernardini*[2], *Alessandro De Luca*[2], *Isabella Torrente*[2], *Antonio Pizzuti*[1]

[1]*Sapienza University of Rome, Rome, Italy,* [2]*Fondazione IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy.*

Introduction: Genome-wide androgenetic mosaicism is a rare condition in which two euploid cell lines coexist in the same individual, one with biparental content and one with genome-wide paternal isodisomy. It can present prenatally resembling Beckwith-Wiedemann syndrome (BWS). We report a fetus with a laborious diagnosis due to discordant results from cultured and uncultured samples.

Materials and Methods: A pregnant was referred at 15 gestational weeks for placental mesenchymal dysplasia and omphalocele. Karyotype, chromosomal microarray analysis (CMA) and BWS molecular testing (methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) analysis of 11p15 BWS critical region) were performed after amniocentesis. These tests were requested again on umbilical cord sample and on previously cultured amniocytes.

Results: Karyotype, CMA performed by an oligonucleotide-array and BWS MS-MLPA after amniocentesis were normal. BWS MS-MLPA from cultured amniocytes and umbilical cord sample showed KvH19 locus hypermethylation and KvDMR hypomethylation in mosaicism. These results, along with microsatellite analysis of BWS region, were consistent with mosaic paternal isodisomy of chromosome 11p15. Analysis performed to assess maternal contamination showed on cultured amniocytes and umbilical blood sample that the paternal alleles were constantly higher on all the microsatellite analyzed mapping in different chromosomes. This result leaded to suspicion of genome-wide androgenetic mosaicism, confirmed via SNP-array analysis from cultured amniocytes, with mosaic rate of 60%.

Conclusions: Assessment of genome-wide androgenetic mosaicism requires multiple laboratory approaches and an extension of the current diagnostic process and caution to low rate of mosaicism. Clinical acumen and an integrated testing approach are the key to a successful diagnosis.

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P01.036.D Sensitive screening of cell free DNA to determine the origin of trophoblastic tumours

Geoffrey J. Maher, *Rosemary A. Fisher, Baljeet Kaur, Xianne Aguiar, Preetha Aravind, Natashia Cedeno, James Clark, Debbie Damon, Ehsan Ghorani, Foteini Kalofonou, Ravindhi Murphy, Rajat Roy, Naveed Sarwar, Mark R. Openshaw, Michael J. Seckl*

Imperial College London, London, United Kingdom.

Introduction: Trophoblastic tumours that secrete human chorionic gonadotropin (hCG) are commonly gestational in origin and are typically diagnosed months to years after the causative pregnancy. However, hCG secretion can also occur in somatic or germ cell tumours. Despite the clinical importance of distinguishing between gestational and non-gestational trophoblastic tumours, which vary in their prognoses and treatment regimens, tumour biopsies are not always available for genotyping due to the risk of haemorrhage. Here we aimed to develop a sensitive cell free DNA (cfDNA) assay to non-invasively determine the origin of hCG-secreting tumours.

Materials and Methods: Genomic DNA and cfDNA from 23 women with hCG-secreting tumours underwent library preparation, probe capture and deep (>5,000x) Illumina sequencing of 195 common autosomal single nucleotide polymorphisms (SNPs) and 13 sex chromosome loci. Gestational tumours were identifiable by the presence of 'non-host' (i.e. paternal) alleles in cfDNA at SNPs that were homozygous in the genomic DNA.

Results: In gestational cases, non-host alleles were detected at multiple SNPs; non-host cfDNA comprised 0.3% - 41.4% of total cfDNA and correlated with serum hCG levels (906 – 3,042,881 IU/ml), although detection was variable below ~1,500 IU/ml. Non-host alleles were not detected in non-gestational cases, but the presence of circulating tumour DNA was confirmed by the identification of copy number alterations.

Conclusions: Previous methods for detecting cfDNA from hCG-secreting tumours lacked sensitivity or were patient-specific, making them unsuitable for routine diagnostic testing. Our sensitive non-invasive assay, applicable to any patient, will facilitate diagnosis at an earlier timepoint and improve patient management.

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P01.038.B Delivering accurate, reproducible non-invasive prenatal diagnosis (NIPD) for sickle cell disease using droplet digital PCR

Joe Shaw[1], *Ben Paternoster*[1], *Maureen Ramos*[1], *Sarah Nesbitt*[1], *Natalie Chandler*[1], *Lyn S. Chitty*[1,2]

[1]*NHS North Thames Genomic Laboratory Hub, London, United Kingdom,* [2]*UCL Great Ormond Street Institute of Child Health, London, United Kingdom.*

Introduction: Sickle cell disease (SCD) is the most common single-gene indication for prenatal diagnosis in England. Non-invasive prenatal diagnosis (NIPD) for SCD is desired by patients, but complicated by the high background of the maternal mutation and frequent unavailability of paternal samples. Droplet digital PCR (ddPCR) offers the potential for NIPD using only a maternal sample via relative mutation dosage; however previous reports describe unacceptable rates (up to 10%) of incorrect genotype classifications.

Methods: A ddPCR assay was designed for the common SCD variant, *HBB* c.20A>T, and validated on heterozygous genomic DNA and paternal cell free DNA. Following optimisation, cell free DNA was extracted from 65 frozen maternal plasma samples from pregnancies at risk of SCD with known fetal genotypes. ddPCR analysis was performed blinded using a modified sequential probability ratio test (SPRT).

Results: ddPCR testing correctly predicted the fetal genotype in 48 cases (32 male, 16 female), with nine of 10 HbSS fetuses correctly identified. A sample from a dichorionic twin pregnancy was included, and the modified SPRT analysis correctly classified one fetus as affected whilst the other was heterozygous. The remaining 17 (26%) cases were inconclusive, with no incorrect fetal genotype predictions.

Conclusion: We have successfully optimised a ddPCR assay that accurately predicts the fetal genotype in pregnancies at risk of SCD. Further optimisation of cfDNA extraction and sampling is required to reduce the rate of inconclusive results and confirm accuracy. Funding was from the GOSH NIHR Biomedical Research Centre. Samples were obtained from the RAPID sample bank.

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P01.039.C Telomeres in TB is longer than in ICM in humans at the blastocyst stage

Anna A. Pendina[1], *Andrei V. Tikhonov*[1], *Vladimir A. Bogomolov*[2], *Mikhail I. Krapivin*[1], *Olga A. Efimova*[1], *Irina D. Mekina*[1], *Evgeniia M. Komarova*[1], *Natalia P. Smirnova*[3], *Olga G. Chiryaeva*[1], *Alexander M. Gzgzyan*[1], *Igor Yu. Kogan*[1], *Vladislav S. Baranov*[1]

[1]*D.O. Ott Research Institute of Obstetrics, Gynecology and Reproductology, St.Petersburg, Russian Federation,* [2]*St. Petersburg State Pediatric Medical University, St.Petersburg, Russian Federation,* [3]*"Mother and Child St.Petersburg", St.Petersburg, Russian Federation.*

Telomeres are complexes of short tandem DNA repeats and proteins at the ends of chromosomes. Their main function is the protection of chromosomes from shortening caused by DNA loss during each replication cycle. Correct regulation of telomere length (TL) is crucial for normal embryogenesis. Here, we performed a pairwise comparison of TLs in trophectoderm (TE) and inner cell mass (ICM) of human blastocysts. A total of 22 blastocysts were included in the study. All the blastocysts were not suitable for transfer because of genetic abnormalities revealed by preimplantation genetic testing. Each blastocyst was dissected into TE and ICM using a microsurgical laser and fixed on a glass slide. Telomeres were detected by qFISH (Telomere PNA FISH Kit/Cy3, Agilent). To avoid possible bias caused by different chromatin condensation, TLs were assessed as relative values by dividing the telomeric fluorescence by the fluorescence of reference region (21q22.13-q22.2) measured in ImageJ 1.51i. The relative TL values ranged between 0.089-0.607 in TE and between 0.061-0.414 in ICM. In TE, the relative TL appeared to be higher than in ICM ($p = 0.029$, paired t-test). Longer telomeres in TE may be linked to its crucial role in implantation and subsequent placentation, both accompanied by a high mitotic activity. Supported by RSF № 18-75-10046.

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P01.040.D Are children born after medical assisted reproduction at greater risk of having an increased *de novo* mutation rate?

*Roos M. Smits[1], Manon S. Oud[2], Aukje M. Meijerink[1], Petra de Vries[2], Giles S. Holt[3], Bilal K. S. Alobaidi[3], Lois Batty[3], Kathrin Fleischer[1,4], Didi D. M. Braat[1], Liliana Ramos[1], **Miguel J. Xavier**[3], Joris A. Veltman[3]*

[1]Department of Obstetrics and Gynaecology, Radboudumc, Nijmegen, Netherlands, [2]Department of Human Genetics, Donders Institute for Brain, Cognition and Behaviour, Radboudumc, Nijmegen, Netherlands, [3]Biosciences Institute, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne, United Kingdom, [4]TFP Center of Reproductive Medicine, Düsseldorf, Germany.

Introduction: *De novo* mutations (DNMs) play a prominent role in sporadic disorders with reduced fitness such as infertility and intellectual disability. Advanced paternal age is known to increase disease risk in offspring by increasing the number of DNMs in their genome. Less is known about the effect of assisted reproduction techniques (ART) on the number of DNMs in offspring. With the on-going trend of delayed parenthood more children are now born both from older fathers and through ART.

Materials and Methods: We investigated 49 trios (mother, father and child) and 2 quartets (mother, father and 2 siblings) divided into children born after spontaneous conception (n = 18); born after in vitro fertilisation (IVF) (n = 17) and born after intracytoplasmic sperm injection combined with testicular sperm extraction (ICSI-TESE) (n = 18). Groups further divided by paternal age, young (<35) or old (>45 years of age at conception). Whole-genome sequencing was performed twice to independently detect and validate all DNMs in children.

Results: A clear paternal age effect was observed, with 70 DNMs detected on average in children born to young fathers and 94 DNMs in those born to older fathers (p = 0.001). No significant differences were observed between different methods of conception (p = 1) with paternal age affecting all methods equally.

Conclusions: Paternal age, not method of conception, had a major effect on the observed number of DNMs in offspring. Given the role DNMs in disease risk, this negative result is good news for IVF and ICSI-TESE born children, if replicated in larger cohorts.

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P01.041.A The relevance of loss-of-function variants in the X-chromosomal gene *TEX13B* in azoospermia is questionable

***Margot J. Wyrwoll**[1,2], Manon S. Oud[3], Matthias Vockel[4], Sabine Kliesch[2], Corinna Friedrich[1], Frank Tüttelmann[1]*

[1]Institute of Reproductive Genetics, University of Münster, Münster, Germany, [2]Centre of Reproductive Medicine and Andrology, Department of Clinical and Surgical Andrology, University Hospital Münster, Münster, Germany, [3]Department of Human Genetics, Radboud University Medical Centre, Nijmegen, Netherlands, [4]Institute of Human Genetics, University of Münster, Münster, Germany.

Introduction: Azoospermia is often assumed to be of genetic origin, with constantly emerging genes in the context of male infertility. Previously, a chromosomal translocation in two infertile brothers with breakpoints close to *TEX13B* was described and a recent publication reported a stop-gain variant in *TEX13B* as cause for azoospermia in one man. According to RNA-sequencing data, *TEX13B* is a germ cell-specific gene with highest expression in spermatogonia. These findings suggest an association of the X-linked gene *TEX13B* with male fertility.

Materials and Methods: Because these studies were limited in size, we screened the exome data of our Male Reproductive Genomics (MERGE) cohort of currently 1,003 men with various infertility phenotypes and some fertile controls for variants in *TEX13B*. Additionally, we utilised the exome data of 5,784 genetically proven fathers. The data were filtered for rare stop-gain, frameshift and splice-site variants (minor allele frequency [MAF] <1% in gnomAD database) in *TEX13B*.

Results: We detected nine azoospermic men carrying the hemizygous stop-gain variant c.382C>T;p.(Gln128Ter) in *TEX13B*. A fertile control, a man with asthenoteratozoospermia and a proven father were also hemizygous for this variant. Another proven father carried the hemizygous splice-donor variant c.459+1G>A in *TEX13B*.

Conclusions: Based on these findings, we question whether pathogenic variants in *TEX13B* are a monogenic cause for azoospermia. We are currently screening additional fertile men for the recurrent stop-gain variant c.382C>T;p.(Gln128Ter) to determine the frequency of this variant in the fertile male population. This work was supported by the DFG Clinical Research Unit 326 'Male Germ Cells'.

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P01.043.C Impact of cigarette smoking on the expression of oxidative stress-related genes in cumulus cells retrieved from healthy women undergoing IVF

***Fani Konstantinidou**[1,2], Maria Cristina Budani[3], Annalina Sarra[4], Gian Mario Tiboni[3], Liborio Stuppia[1,2], Valentina Gatta[1,2]*

[1]School of Medicine and Health Sciences, "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy, [2]Unit of Molecular Genetics, Center for Advanced Studies and Technology (CAST), "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy, [3]Department of Medical, Oral and Biotechnological Sciences, "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy, [4]Department of Philosophical, Pedagogical and Quantitative Economic Sciences, "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy.

Increased production of reactive oxygen species is an important factor in the pathophysiology of fertility decline. Cigarette smoking, for example, can directly derange folliculogenesis by ROS production. Delicate communication between the germline and cumulus cells (CCs) is also the basis for all processes in ovarian physiology. Several studies have aimed to discover non-invasive tools to indirectly evaluate oocyte quality by focusing on CCs as a mirror of oocyte characteristics. However, the general lack of uniformity among them indicates that their utility in the clinical setting remains controversial due to their high sensitivity to intrinsic and extrinsic factors related to the patient.On these premises, we focused our attention on evaluating the impact of tobacco smoking on gene expression of CCs in two categories of individuals, 5 overall healthy smokers and 5 non-smokers (<35 years of age) undergoing IVF treatments. Total RNA was extracted from CCs of all subjects and subsequently reverse transcribed. Predesigned oxidative stress 96-well plates were used to perform qRT-PCR analysis. A gene was considered differentially expressed in smokers' CCs versus control CCs when showing a fold change >1.4 or <0.7 and a P-value < 0.05 (ANOVA). Statistical analysis showed a significant down-regulation of genes protecting and repairing cells against oxidative damage in CCs of all or the majority of smoking females compared to their corresponding age-matched controls, indicating a cigarette smoke-derived impact on the oxidative stress pathway.In conclusion, an important interaction between cigarette smoking and oxidative stress-related genes in possible fertility biomarkers is clearly evidenced.

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P01.044.D To be or not to be pathogenic - distinguishing pathogenic mutations from benign missense variants in *NR5A1*

Jana Emich*[1], Corinna Friedrich*[1], Christina Burhöi[1], Thaís Fenz Araujo[2], Ann-Christin Tewes[3], Susanne Ledig[3], Albrecht Röpke[3], Sabine Kliesch[4], Jörg Gromoll[5], Frank Tüttelmann[1]

[1]Institute of Reproductive Genetics, University of Münster, Münster, Germany, [2]Department of Genetics, Ribeirao Preto Medical School, University of Sao Paulo, Ribeirao Preto, Brazil, [3]Institute of Human Genetics, University of Münster, Münster, Germany, [4]Centre of Reproductive Medicine and Andrology, Department of Clinical and Surgical Andrology, University Hospital Münster, Münster, Germany, [5]Centre of Reproductive Medicine and Andrology, Institute of Reproductive and Regenerative Biology, University of Münster, Münster, Germany.

*These authors contributed equally.

Introduction: Infertility affects 10-15% of all couples. The most severe form of male infertility is azoospermia, of which most cases are suspected to be of genetic origin. The transcription factor SF1, encoded by *NR5A1*, plays a central role in gonadal development. Severe variants are associated with gonadal dysgenesis and sex reversal, while mild missense variants have been reported in isolated forms of male infertility.

Materials and methods: The exome data of 1,003 otherwise healthy infertile men from the Male Reproductive Genomics (MERGE) cohort was filtered for heterozygous missense variants in *NR5A1* with a minor allele frequency of ≤0.1% in the gnomAD database. The identified variants were forwarded to functional analysis by site-directed mutagenesis and a luciferase reporter assay.

Results: We detected eight potentially relevant missense variants in nine patients with severe oligozoospermia or azoospermia, resulting in a detection rate of 1.0% for *NR5A1* in the MERGE cohort. In the luciferase assay, two of these variants showed reduced SF1 transcriptional activity.

Conclusions: The assessment of missense variants is challenging, warranting functional analyses to distinguish pathogenic mutations from benign variants. The luciferase reporter assay supports that two of the investigated variants are relevant in the pathogenesis of spermatogenic failure and male infertility.

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P01.045.A FKBP6 has an essential role in human spermatogenesis

Godfried W. van der Heijden*[1,2], Manon Oud[2], Rytis Stakaitis[3], Ieva Golubickaite[3], Channah Gaasbeek[2], Nadja Rotte[4,5], Sabine Kliesch[4], Liliana Ramos[1], Kristian Almstrup[3], Miguel J. Xavier[6], Joris A. Veltman[6], Frank Tüttelmann[5]

[1]Division of Reproductive Medicine, Department of Obstetrics and Gynecology, Radboud University Medical Centre, The Netherlands, Nijmegen, Netherlands, [2]Department of Human Genetics, Donders Institute for Brain, Cognition and Behavior, Radboud University Medical Centre, Nijmegen, Netherlands, [3]Copenhagen University Hospital - Rigshospitalet, Department of Growth and Reproduction, Copenhagen, Denmark, [4]Centre of Reproductive Medicine and Andrology, Department of Clinical and Surgical Andrology, University of Münster, Münster, Germany, [5]Institute of Reproductive Genetics, University of Münster, Münster, Germany, [6]Biosciences Institute, Faculty of Medical Sciences, Newcastle University, Newcastle, United Kingdom.

We performed exome sequencing in an infertile patient and his fertile parents to identify the genetic cause of his germ cell arrest in spermatogenesis. A homozygous pathogenic 22-bp insertion was identified in the *FKBP6* gene. In an independent cohort, a second patient with a different homozygous variant predicted to be pathogenic was also identified in *FKBP6*. RNA isolated from testicular tissue was used to show that *FKBP6* expression levels were severely reduced in both patients, confirmed by immunostaining. In male mice, *Fkbp6* functions in the fetal piRNA-pathway and localizes to the synaptonemal complex (SC) during meiosis. In adult male KO mice, failure to complete chromosome synapsis causes meiotic arrest, presumably due to its absence from the SC. To ascertain if these features also underlie infertility in humans, we analyzed meiotic progression in the two cases. Germ cell loss did occur during meiosis but not due to incomplete synapsis of the chromosomes. In control samples, we were unable to detect FKBP6 at the SC, altogether suggesting a function for FKBP6 outside SC-biology. To determine if a role for FKBP6 in the piRNA-pathway was evident in humans, we assessed its co-localization with piRNA-pathway factors PIWIL1 and TDRKH. In controls, FKBP6 localized to cytoplasmic granules together with PIWIL1 and TDRKH. Loss of FKBP6, however, did not affect the localization of these factors. Our data indicates that *FKBP6* is required for human spermatogenesis and that it might play a role in the piRNA-pathway rather than in the establishment of the SC.

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P01.047.C Effects of polymorphisms of energy metabolism and angiogenesis genes on intrauterine growth restriction

Irina Novikova*, Inna Pokudina, Elena Mashkina, Tatiana Shkurat

Southern Federal University, Rostov-on-Don, Russian Federation.

Introduction: Intrauterine growth restriction (IUGR) is a condition in which the growth rate of the fetus during pregnancy is less than expected. Energy metabolism genes play an important role in the regulation of both maternal and fetal body weight and thus in the development of IUGR. Another placental key factor of IUGR physiopathology is angiogenesis. The aim of research is to study the polymorphisms of genes of energy metabolism (*LEPR*; *FTO*) and angiogenesis (*NOS3*; *VEGFA*) in women with IUGR compared to the control group to identify it`s possible functional significance in the pathogenesis of IUGR.

Materials and Methods: The material for the study was blood samples from 36 pregnant women diagnosed with IUGR and 30 healthy women. Genotyping of targeted SNPs: *A223G* (*LEPR*), *A23525T* (*FTO*), *C786T* (*NOS3*), *C634G* (*VEGFA*) were detected by RT-PCR.

Results: No statistically significant associations of *LEPR* and *FTO* gene polymorphisms with IUGR were found ($p = 0.765$; $p = 0.461$, respectively). Genotypes *CT* (*NOS3*) and *CC* (*VEGFA*) were significantly associated with low risk of developing IUGR. OR were 0,309 (CI 95% 0,110-0,868 $p < 0.001$) and 0.14 (CI 95% 0.04-0.46 $p = 0.003$) respectively. Genotypes *TT* (*NOS3*) and *GG* (*VEGFA*) were associated with higher relative risk of developing IUGR. OR were 2,400 (CI 95% 1,462-3,339 $p < 0,001$) and 3.67 (CI 95% 1.26-10.7 $p = 0.003$) respectively.

Conclusions: The data obtained indicate the need for further investigation to search for genetic determinants of the development of IUGR. This study was funded by the Ministry of Science and Higher Education of the Russian Federation #0852-2020-0028

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P01.048.D KIR-HLAC genotyping in married couples with unexplained early reproductive losses

Kateryna Sosnina, *Danuta Zastavna, Oresta Terpyliak, Bogdan Tretiak*

Institute of Hereditary Pathology, NAMS of Ukraine, Lviv, Ukraine.

The immune interaction between the uterus and the embryo is crucial to achieve a pregnancy. Maternal immunotolerance is provided by the interaction between the cells of the maternal immune system in the uterus (uterine NK cells) and the embryo. These cells recognize the embryo through KIR receptors, interacting with their HLA-C ligands on the embryo. The aim of this study was to establish and analyze the combinations of *KIR* and *HLA-C* genes in married couples with unexplained early reproductive losses.

Results: *KIR-HLAC* genotyping was performed in 43 married couples with unexplained reproductive losses. Depending on the number and type of genes, the *KIR* genotype of women (*AA, AB* and *BB*) was established. The results were compared with the parental *HLAC* genotype, and the risk of reproductive losses in such a pair was calculated. According to the results, 23.3% of women have the *AA* genotype (high risk of reproductive loss), 76.7% of women had the *AB* genotype (medium risk). According to the results of *KIR-HLAC* genotyping of couples with early reproductive losses, 58.14% of married couples have a high risk of reproductive losses. Such married couples had the *KIR AA* genotype in women and/or the *C2/C2 HLAC* genotype in women and/or men.

Conclusions: *KIR-HLAC* genotyping is a genetic test that allows to assess the risks of the embryo being rejected by the maternal immune system, and thus to direct medical interventions in order to achieve a successful pregnancy.

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P01.050.B Identification of two novel point mutations in *DPY19L2* leading to total globozoospermia in two unrelated patients

Fatma Abdelhedi[1,2], *Emmanuel Dulioust*[3], *Nourhène Gharbi*[2], *Sandrine Barbaux*[4], *Ahmed Ziyyat*[4], *Leila KESKES*[2], *Jean Michel Dupont*[1,4]

[1]*Laboratoire de Cytogénétique, Hôpital Cochin, APHP, Paris, France,* [2]*Laboratoire de Génétique Moléculaire Humaine, Faculté de Médecine, Sfax, Tunisia,* [3]*Laboratoire d'Histologie Embryologie Biologie de la Reproduction, Hôpital Cochin, APHP, Paris, France,* [4]*Génomique, Epigénétique et Physiopathologie de la Reproduction, U1016 INSERM-UMR 8104 CNRS, Institut Cochin, Université Paris Descartes, Paris, France.*

Background: Globozoospermia is a rare and severe teratozoospermia characterized by the presence of round-headed spermatozoa lacking an acrosome. So far, only three genes have been well documented as responsible for this phenotype in human, namely *DPY19L2, SPATA16*, and recently *GGN*. Genetic causes remain unexplained for 20% to 30% of patients with globozoospermia suggesting that this phenotype is likely genetically heterogeneous.

Methods: As alterations of *DPY19L2* gene represent the main cause of globozoospermia in human, we first screened for *DPY19L2* molecular defects in a cohort of 10 patients of African and European origin, diagnosed with complete or partial globozoospermia. Whole genome analysis with SNP array was carried out in patients lacking genetic defects of *DPY19L2*.

Results: Molecular analyses performed on 9 genetically independent individuals showed that four (44%) were homozygous for the DPY19L2 deletion, two were heterozygous composite for two novel non-synonymous mutations (p.R686G and p.D687E) in exon 21. No genetic causes were identified in four patients with partial globozoospermia.

Conclusion: Our results highlight the importance of screening for *DPY19L2* mutations in the absence of *DPY19L2* deletions and strongly suggest that partial globozoospermia is not due to genetic defects on *DPY19L2*. Alghough the genetic diagnosis of globozoospermia does not yet provide any clear therapeutic indications, a molecular diagnosis remains useful to provide adequate genetic counseling. Considering these points, we recommend the search for *DPY19L2* defects as the first-line genetic analysis in complete globozoospermia but not in its partial form.

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P01.051.C Causal inference analyses reveal population risks and benefits of extending female reproductive lifespan through manipulation of biological processes

Katherine S. Ruth[1], *Felix R. Day*[2], *Anna Murray*[1], *John R. B. Perry*[2], *ReproGen consortium (www.reprogen.org)*

[1]*Genetics of Complex Traits, University of Exeter Medical School, University of Exeter, Exeter, United Kingdom,* [2]*MRC Epidemiology Unit, University of Cambridge School of Clinical Medicine, Institute of Metabolic Science, Cambridge, United Kingdom.*

Introduction: Recently we demonstrated the potential for new therapeutics to extend female reproductive lifespan by manipulating DNA damage response (DDR) pathways (MedRxiv https://doi.org/10.1101/2021.01.11.20248322). We investigated the wider population health risks and benefits of prolonged sex hormone exposure from such interventions.

Materials and Methods: We performed Mendelian Randomization using a genetic instrument for age at menopause (ANM) comprised of 290 variants from GWAS in >200,000 women. We tested outcomes associated with hormone replacement therapy, as being analogous to longer hormone exposure from later ANM.

Results: One-year later genetically-instrumented ANM increased the odds of female-specific hormone-sensitive cancers by up to 5%. We identified individual variants with effects on DDR and cancer risk independently of ANM and sex hormone exposure - loss-of-function alleles in *BRCA2* and *CHEK2* resulted in ANM 1.69 years earlier (95% CI 0.12-3.26, $p = 0.03$) and 2.36 years later (1.31-3.41, $p = 1 \times 10^{-5}$). We used MR-Radial to identify and exclude such variants from analyses, strengthening the evidence for an association with ER+ breast cancer (OR = 1.04, from $p = 4 \times 10^{-9}$ to $p = 3 \times 10^{-16}$). Later ANM had protective effects on Type 2 diabetes (OR = 0.98, $p = 1 \times 10^{-3}$) and bone health (fracture, OR = 0.98, $p = 3 \times 10^{-4}$). There was little evidence for associations with cardiovascular disease, longevity or Alzheimer's disease.

Conclusions: Later ANM is detrimental for hormone sensitive cancers yet benefits metabolic and bone health, consistent with

effects of oestrogen therapy in trials. However, our results do not support associations from observational studies. Novel therapeutic approaches to extending reproductive lifespan are likely to have wider effects on population health over prolonged fertility.

K.S. Ruth: None. **F.R. Day:** None. **A. Murray:** None. **J.R.B. Perry:** None.

P01.052.D Locus-specific methylation of the *ESR1* gene promoter region as a marker for the prognosis of placental insufficiency and perinatal loss

Zoia I. Rossokha[1], *Ludmila I. Vorobey*[2], *Valeriy V. Kaminskyi*[2], *Tetjana V. Kolomiichenko*[2], *Llia Y. Fishchuk*[1], *Natalia L. Medvedieva*[1], *Ludmila I. Brisevac*[2], *Olexandr I. Zhdanovych*[2], *Natalia G. Gorovenko*[2]

[1]*State Institution "Reference-centre for molecular diagnostic of Public Health Ministry of Ukraine", Kyiv, Ukraine,* [2]*Supic Medical Acadeny of Postgraduate Education, Kyiv, Ukraine.*

Introduction: Chronic stress and polluted environment are known direct determinants in prenatal maternal promoter hypermethylation of *ESR1* gene and blockade of estrogen-dependent processes in the placenta. *ESR1* gene expression is important for embryo implantation and pregnancy outcome, neuroprotection throughout life from antenatal period, and also has an organizational impact on the formation of long-term behavioral and cognitive functions, as well as maintaining energy homeostasis. The aim of the study was to investigate the effect of hypermethylation of the *ESR1* gene promoter region on the risk of early pregnancy and perinatal loss.

Materials and Methods: 31 women with pregnancy loss and 10 women without reproductive failure were involved in the study. 24 women had early pregnancy loss and 7 women had perinatal loss. Methylation of the promoter region of the *ESR1* gene was determined by methyl-specific PCR using DNA after bisulfide conversion.

Results: Hypermethylated *ESR1* promoter region was detected in 35,48% patients with pregnancy loss. 29,16% patients with early pregnancy loss had hypermethylated status in *ESR1*. More often hypermethylated status was found among patients with perinatal loss - 57,14%. And no hypermethylated cases were detected in women without reproductive failure. Determination of hypermethylated *ESR1* promoter region was significantly higher in patients with perinatal loss and 75% of them had had placental insufficiency resulting in antenatal death at 26-28 weeks of gestation.

Conclusion: Hypermethylation of the *ESR1* gene promoter region in women was associated with placental insufficiency and perinatal loss. Further research is needed to develop personalized methods of perinatal loss prevention.

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P01.053.A The role of miR 196b in the pathogenesis of endometriosis

Victoria Spasova[1], *Vesela Karamisheva*[1,2], *Olga Antonova*[1], *Zora Hammoudeh*[1], *Liliya Koleva*[3], *Rada Staneva*[1], *Mihail Ganev*[1], *Desislava Nesheva*[1], *Draga Toncheva*[1], *Savina Hadjidekova*[1]

[1]*Medical University of Sofia, Sofia, Bulgaria,* [2]*University obstetrics and gynecology hospital "Maichin dom", Sofia, Bulgaria,* [3]*University Hospital "Pirogov", Sofia, Bulgaria.*

Introduction: Endometriosis is a chronic disease affecting about 10% of women worldwide. There is still a discussion among scientist on the exact molecular pathogenesis of the disease appearance. MiRNAs as posttranscriptional expression regulators may contribute to the development of endometriosis.

Materials and methods: Total RNA was isolated from tissue samples of ectopic endometrium of 30 patients with endometriosis and healthy endometrium of 15 controls (miRNeasy-MiniKit, Qiagen). Three pool samples were constructed based on the disease stage - each containing 15 RNA samples: early stage endometriosis, late stage endometriosis, and controls. Reverse transcription was conducted on each of the pool samples via miScript-II-RT Kit,Qiagen. SYBR-Green-based Real-time PCR assay was used to determine the expression profile of 84 miRNAs (Human miFinder miRNA PCR Array,Qiagen). QIAGEN's GeneGlobe Data Analysis Centre was used to perform the data analysis. The prediction of target genes was accomplished using miRBase.org, Targetscan.org and Diana tools.

Results: We detected miR-196b-5p downregulated in both stages of endometriosis compared to the healthy controls (fold change = -19,28 for the early stage, fold change = -139,54 for the late stage). HOX genes are a known target of miR-196b-5p. These genes encode proteins that act as transcription factors. Changes of HOX genes expression are associated with altered endometrium development.

Conclusion: We suggest that the altered expression of miR-196b may contribute to reveal the etiology of endometriosis occurrence and progression.Funding. Contract No.212/12.12.2018, grant No.4731/03.07.2018, Council of Medical Science at the Medical University of Sofia, Bulgaria; Bulgarian Ministry of Education and Science, National Program for Research "Young Scientists and Postdoctoral Students."

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P01.054.B Comparative analysis of cytogenetic abnormalities revealed by karyotyping and interphase FISH in chorion of first-trimester miscarriages

Andrei V. Tikhonov[1], *Olga A. Efimova*[1], *Mikhail I. Krapivin*[1], *Yanina M. Sagurova*[1], *Anna A. Pendina*[1], *Alla S. Koltsova*[1], *Anna A. Smirnova*[2], *Olga E. Talantova*[1], *Olga G. Chiryaeva*[1], *Lubov' I. Petrova*[1], *Vera S. Dudkina*[1], *Vladislav S. Baranov*[1]

[1]*D.O. Ott Research Institute of Obstetrics, Gynecology and Reproductology, St.Petersburg, Russian Federation,* [2]*St. Petersburg State Pediatric Medical University, St.Petersburg, Russian Federation.*

Abnormal karyotype is a frequent cause of first-trimester miscarriage. In ~20% of cases conventional karyotyping is not possible because of absence of mitotic activity in chorion. Here, we compared the results of karyotyping and interphase fluorescence in situ hybridization (FISH) with centromeric/locus-specific DNA-probes specific to all chromosomes excluding chromosomes 1 and 19 (Abbott Molecular) on chorion of first-trimester miscarriages. A total of 3511 cases were retrospectively analyzed. In 2816 cases, conventional karyotyping was performed on direct metaphase preparations. In 695 cases, interphase FISH was performed because of no metaphases on direct preparations. The frequency of abnormal karyotype reached 64.4%(1814/2816) in the karyotyped group and 57.7%(401/695) in the FISH-analyzed group. Expectably, structural chromosomal abnormalities were detected only in the karyotyped group at the frequency of 2.9% (53/1814). The spectrum of revealed numerical karyotype

abnormalities did not differ between the groups. However, their frequencies differed between the karyotyped and FISH-analyzed groups. Aneuploidies were revealed with higher frequency in the karyotyped compared to the FISH-analyzed group: 73.4%(1332/1814) vs 63.8% (256/401) ($p = 0.0001$). In contrast, polyploidy and mosaicism were more frequent in FISH-analyzed compared to the karyotyped group: 26.2% (105/401) vs 16.9% (306/1814) ($p < 0.0001$) and 8.0% (32/401) vs 4.4% (80/1814) ($p = 0.0047$). Thus, FISH can be used for the detection of numerical chromosomal abnormalities in chorion of first-trimester miscarriages, when karyotyping is not possible. Difference in frequencies of the same cytogenetic categories between the samples with and without mitotic activity suggests that viability of chorionic cell depends on the type of chromosomal abnormality. Supported by RSFN№19-75-00023.

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P01.055.C Case report: Prenatal mosaic trisomy 9 as result of a meiotic non-disjunction event

Sebastian Rusch[1,2], *Melanie Gass*[2], *Sevgi Tercanli*[3], *Isabel Filges*[2,4]

[1]*Medical Genetics, Institute of laboratory medicine, Kantonsspital Aarau, Aarau, Switzerland,* [2]*Medical Genetics, Institute of Medical Genetics and Pathology, University Hospital Basel, University of Basel, Basel, Switzerland,* [3]*Centre for Prenatal Ultrasound, Basel, Switzerland, Basel, Switzerland,* [4]*Department of Clinical Research, University Hospital Basel, Basel, Switzerland.*

Introduction: Prenatal mosaic aneuploidy remains a challenge for counseling clinicians. The impact on embryonic and fetal development is depending on the level of mosaicism and tissue distribution of the aneuploid cell line; hence, clinical consequences are difficult to predict. We present the rare instance of mosaic trisomy 9 in a fetus with multiple malformations. We discuss how CMA-SNP data can contribute to understand the mechanism of aneuploidy.

Methods: Prenatal sonography of a 44-year-old woman at 14 weeks of gestation (WG) showed tricuspid regurgitation, a small branchial cyst, single umbilical artery and retrognathia, at 19 WG additional heart, renal and brain anomalies. FTT found increased risk scores of 1:102 and 1:2 for trisomy 21 and 13/18 respectively. CMA was used on amniotic fluid cells to detect copy number variants.

Results: The CMA result on amniotic fluid cells was in respect to copy number state (2.5) and B-Allele Frequencies (BAF) of approx. 0, 40, 60 and 100% concordant with mosaic trisomy 9 of about 50%. Additionally, with BAF of 20 and 80% in several chromosomal regions of chromosome 9 SNP-data signals indicated the presence of an additional haplotype.

Conclusions: CMA-SNP data reveal regions containing an additional haplotype which most likely arose from meiotic crossing over events. The distribution of the additional haplotype in the centromeric region suggests a meiotic I non-disjunction event followed by a postzygotic trisomic rescue for the normal cell line. We increase our knowledge on the fetal phenotype caused by the aberrant cell line which is only rarely reported.

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P01.056.D Mosaicism for copy number variations in the placenta is even more difficult to interpret than mosaicism for whole chromosome aneuploidy

Ida Charlotte Bay Lund[1,2], *Naja Becher*[1,2,3], *Jesper Graakjaer*[4], *Dorte Lildballe*[4], *Niels Uldbjerg*[5,6], *Pauline Bogaard*[7], *Astrid Petersen*[7], *Else Marie Vestergaard*[8], *Ida Vogel*[1,2,6]

[1]*Department of Clinical Genetics, Aarhus University Hospital, Aarhus N., Denmark,* [2]*Center for Fetal Diagnostics, Aarhus University Hospital, Aarhus N., Denmark,* [3]*Department of Biomedicine, Aarhus University, Aarhus N., Denmark,* [4]*Clinical Genetics, University Hospital of Southern Denmark, Sygehus Lillebaelt, Vejle, Denmark,* [5]*Department of Obstetrics and Gynaecology, Aarhus University Hospital, Aarhus N., Denmark,* [6]*Department of Clinical Medicine, Aarhus University, Aarhus N., Denmark,* [7]*Department of Pathology, Aalborg University Hospital, Aalborg, Denmark,* [8]*Department of Clinical Biochemistry, Horsens Regional Hospital, Horsens, Denmark.*

Objective: To compare mosaicisms in prenatal chorionic villus samples with corresponding postpartum placental samples.

Method: We collected placentas from 15 consecutive cases of mosaicism detected in chorionic villus samples and obtained five standardized samples on each placenta after delivery. All pre- and postnatal placental samples were uncultured and analyzed by high-resolution chromosomal microarray.

Results: Ten cases of mosaicism for whole chromosome aneuploidy(mWC) and five cases with mosaicism for (sub) chromosomal copy number variations(mCNVs) were included. In 5/10 mWC cases and in 4/5 mCNV cases the prenatally detected aberration was confirmed in the postpartum placenta. Three postpartum placentas revealed various complex aberrations differing from the prenatal results: 1) mosaicisms for different deletions/duplications on 9p and 9q in all samples (prenatal: mosaic 5.3 Mb duplication on 9p24), 2) different regions with deletions/duplications/loss of heterozygosity on 1p in all samples (prenatal: mosaic 2.3 Mb 1p36 duplication), and 3) mosaicism for a duplication on 5q and a deletion on 6p in one out of five samples (prenatal: mosaic trisomy 7).

Conclusion: CNVs constitute a complex subgroup in placental mosaicism. Counseling of these couples after chorionic villus sampling shouldn´t focus on the specific CNV involved, but on the nature of mosaicism and the option of amniocentesis and ultrasound.

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P01.057.A Prenatal diagnosis of Neu-Laxova syndrome through identification of a novel exonic-deletion and missense mutation in the *PHGDH* gene

Aruna Marchetto[1], *Moneef Shoukier*[1], *Katja Gahle*[1], *Anne Janke*[1], *Justin Seidel*[1], *Ursula Hiener*[2], *Vera Link*[3], *Kai Wagner*[3], *Sabine Minderer*[1], *Karl Philipp Gloning*[1]

[1]*Prenatal-Medicine Munich, Munich, Germany,* [2]*Klinikum Dritter Orden - Department of Radiology, Munich, Germany,* [3]*Rotkreuzklinikum - Institute of Pathology, Munich, Germany.*

Mutations in the *PHGDH* gene disrupt the serine biosynthesis and are known to be associated with the autosomal recessive disorder Phosphoglycerate dehydrogenase deficiency (PHGDH) and the more severe form Neu-Laxova syndrome 1 (NLS1). To date, there is no clear genotype-phenotype correlation between PHGDH and

NLS1. PHGDH is characterized by congenital microcephaly, psychomotor retardation and seizures whereas it has been assumed that NLS1 could be caused by more severe *PHGDH*-mutations with main clinical features of intrauterine growth retardation (IUGR), microcephaly, cutaneous and craniofacial abnormalities. We present a case of a prenatal conspicuous microcephaly linked to two novel mutations in the *PHGDH* gene likely associated with NLS1.

We report on a pregnant 42-year-old patient, 23rd gestational week, whose fetus presented with severe microcephaly, IUGR, cleft lip, dilated intestinal loops and mild hydronephrosis. MRI confirmed sonographic findings while array-CGH was normal (arr (1-22)x2,(XY)x1). A multigene panel analysis (brain malformations panel, 400 genes) revealed two likely disease-causing variants; a maternal inherited hemizygous missense mutation c.766G>A, p. Gly256Arg in exon 7 and a paternal inherited heterozygous deletion of exon 6 and 7 of the PHGDH gene. The pregnancy was terminated in the 25th week. Immunohistological staining of the fetuses' skin biopsy revealed ichthyosis and hyperkeratosis. Both detected mutations in the PHGDH gene were localized in the nucleotide-binding domain (NBD).

Considering the molecular and clinical findings, we assume these mutations to be causative for a Neu-Laxova syndrome. Thus, our results support previous findings indicating that mutations in NBD mostly cause the more severe phenotype of PHGDH-associated disorders (NLS1).

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P01.058.B Neurofibromatosis type 1 and the next generation: is preimplantation genetic testing the solution?

Vivian Vernimmen, *Aimee D. C. Paulussen, Constance T. R. M. Stumpel, Ron J. T. van Golde, Christine E. M. de Die*

Maastricht University Medical Center, Maastricht, Netherlands.

Introduction: Future parents affected with Neurofibromatosis type 1 (NF1) can opt for preimplantation genetic testing (PGT) to avoid NF1 in their offspring. We aim to identify challenges and pitfalls in PGT for NF1.

Methods: We collected data on PGT cycles from the medical files of couples requesting PGT for NF1 between January 1997 and January 2020.

Results: PGT was the reproductive option of choice for 96 couples. PGT was not possible for 14 couples, mostly because the causative variant was not identified or of unknown significance. The origin of the *NF1* mutation was (presumed) sporadic in 63% of the 82 couples proceeding with PGT. PCR with multiple polymorphic markers was most frequently applied, combined with direct mutation analysis if needed. PGT/PCR work-up showed several exceptional situations: in one family two different *NF1* mutations turned out to be causal and in one family with a sporadic male index the mutation was not detected in the sperm cells during PGT work-up. The 'OnePGT' genome wide haplotyping method, based on next generation sequencing, was used for 2 recent families with a familial mutation. A successful PGT test could be developed for 78 couples with 71 different variants in the *NF1* gene. Together, 65 couples underwent 141 PGT cycles and the transfer of 162 unaffected embryos resulted in 39 ongoing pregnancies (pregnancy rate 24,1%/embryotransfer).

Conclusions: PGT is a successful reproductive option for couples with NF1. Test development was possible in almost all cases reviewed despite the fact that most variants were unique and sporadic.

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P01.059.C Prenatal diagnostics of NF2 mutation in the fetus, associated with the development of endometrial cancer

Kalina Belemezova[1,2], *Kunka Kamenarova*[3], *Kalina Mihova*[3], *Mariela Hristova-Savova*[1], *Albena Todorova*[4], *Radka Kaneva*[3], *Ivanka Dimova*[1,3]

[1]*Genetic Laboratory, SAGBAL Dr. Shterev, Sofia, Bulgaria,* [2]*Medical University of Sofia, Sofia, Bulgaria,* [3]*Molecular Medicine Center, Medical University of Sofia, Sofia, Bulgaria,* [4]*Laboratory Genika, Sofia, Bulgaria.*

Endometrial carcinoma (EC) is rarely diagnosed during pregnancy. An electronic search of the literature revealed just 25 cases of EC diagnosed during or after pregnancy, for the period between 1995 and 2019. Ten percent of endometrial carcinomas harbor *NF2* mutations. This observation is of interest since mutated NF2 can activate mTOR, the downstream effector of PIK3CA, and in a high percentage of this cancer type (>80%), there are PI3K pathway aberrations. *NF2* is a complex gene with somatic mutations being associated with various cancers. Germline mutations cause the autosomal dominant disorder neurofibromatosis 2 (NF2). Individuals with hereditary NF2 do develop schwannomas, ependymomas, and meningiomas. Although the somatic mutations sometimes overlap with those in hereditary NF2, no published papers are documenting an increased risk of other cancer types in neurofibromatosis patients. Here we report a young pregnant woman, affected by classical NF2 (presence of bilateral schwannomas) and carrying mutation c.592C>T (p.Arg198Ter) in exon 6 of the *NF2* gene. Because of the high risk for inheritance (50%), CVS was performed and DNA from the fetus was analyzed for the presence of mutation with Sanger sequencing - the fetus was found to be a carrier of the same pathogenic mutation. In the meantime, ultrasound examination suspected tumor formation in the uterus, which was confirmed to be endometrial cancer after biopsy and pregnancy was terminated. This case suggests an adverse effect of the simultaneously NF2-affected fetus and mother for endometrial malignancy and suggesting PGD when the mother is affected by NF2.

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P01.060.D Patient-friendly integrated first trimester screening by NIPT and fetal anomaly scan

Karin E. M. Diderich, *Malgorzata I. Srebniak, Maarten F. C. M. Knapen, Marieke Joosten, Sander Galjaard, Diane Van Opstal*

ErasmusMC, Rotterdam, Netherlands.

Many major structural fetal anomalies can be diagnosed by first trimester fetal anomaly scan. NIPT can accurately detect aneuploidies and large chromosomal aberrations in cfDNA in maternal blood plasma. This study shows how a patient-friendly first trimester screening for both chromosomal and structural fetal anomalies in only two outpatient visits can be provided. Genotype-first approach assures not only the earliest diagnosis of trisomy 21 (the most prevalent chromosome aberration), but also completion of the screening at 12–14 weeks. To ensure proper management and avoid unnecessary anxiety abnormal

NIPT different from trisomy 21, 18 and 13 should be referred for genetic counseling.

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P01.061.A NIPD for translocation carriers - yes please or no go?

Malgorzata I. Srebniak[1], *Fernanda S. Jehee*[1], *Marieke Joosten*[1], *Marjan Boter*[1], *Walter de Valk*[1], *Robert van der Helm*[1], *Erik Sistermans*[2], *Els Voorhoeve*[2], *Shama Bhola*[2], *Mariette Hoffer*[3], *Nicolette den Hollander*[3], *Merryn Macville*[4], *Diane Van Opstal*[1]

[1]*Erasmus MC, Clinical Genetics, Rotterdam, Netherlands,* [2]*Amsterdam UMC, Vrije Universiteit Amsterdam, Clinical Genetics, Amsterdam, Netherlands,* [3]*Leiden University Medical Center, Clinical Genetics, Leiden, Netherlands,* [4]*Clinical Genetics, GROW School for Oncology and Developmental Biology, Maastricht University Medical Center, Maastricht, Netherlands.*

The presence of an unbalanced familial translocation can reliably be assessed in the cytotrophoblast of chorionic villi. However, carriers of a balanced translocation often refuse invasive testing due to the miscarriage risk. Because the cytotrophoblast is also the source of cell free (cf) DNA in plasma, this study aimed to investigate whether an unbalanced translocation can also be diagnosed in cfDNA by whole-genome NIPT.Pregnant women carrying a fetus with an unbalanced familial translocation, from whom NIPT as well as microarray data were available, were included in this retrospective assessment. NIPT was performed in the course of the TRIDENT study, but at the time of screening, carrier status of a parental translocation was unknown (exclusion criterion for NIPT in the Netherlands). In 12 cases both NIPT and microarray data were available. In 10/12 cases the unbalanced translocation was correctly identified without prior knowledge on parental translocation. One was missed due to too low fetal fraction. One was missed due to technical restrictions in calling 16p gains. Both missed cases were later identified by invasive diagnostic testing after ultrasound anomalies were found.This study supports the hypothesis that routine NIPT may be used for prenatal diagnosis of unbalanced inheritance of familial translocations, especially with prior knowledge of the translocation allowing focused examination of the involved chromosomal regions. Our study showed that routine shallow sequencing designed for aneuploidy detection in cfDNA may be sufficient for higher resolution NIPT, if specialized copy number software is used and if sufficient fetal fraction is present.

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P01.062.B Non-invasive prenatal diagnosis of paternally-inherited disorders: three years clinical experience

Mathilde Pacault[1,2], ***Camille Verebi***[1], *Maureen Lopez*[1], *Nicolas Vaucouleur*[1], *Lucie Orhant*[1], *Nathalie Deburgrave*[1], *France Leturcq*[1], *Dominique Vidaud*[1], *Emmanuelle Girodon*[1], *Thierry Bienvenu*[1], *Juliette Nectoux*[1]

[1]*Service de Génétique et Biologie Moléculaires, Hôpital Cochin, APHP. Centre Université de Paris, Paris, France,* [2]*Laboratoire de Génétique Moléculaire et d'Histocompatibilité, Centre Hospitalier Régional Universitaire, Brest, France.*

Introduction: Cell-free fetal DNA analysis is now performed routinely for aneuploidy screening, fetal RhD genotyping or sex determination. However, applications to single gene disorders (SGD) remain sparse. We previously described a standardized protocol for non-invasive exclusion of paternal mutation in SGD using a targeted, droplet digital PCR-based approach. Here, we report our three-year clinical experience since offering this service.

Patients and Methods: Patients were referred by clinicians nationwide for several monogenic disorders, paternally-inherited or *de novo*, dominant or recessive, such as cystic fibrosis, neurofibromatosis type I or *FGFR3*-related disorders (thanatophoric dysplasia and achondroplasia). Non-invasive prenatal diagnosis was performed using custom assays for droplet digital PCR. Results, turn-around time and cost-effectiveness were evaluated.

Results: Our tests proved very robust and highly discriminant, as a result was obtained for every 205 referral except one, where fetal fraction was too low to conclude, but returned « unaffected fetus » on a subsequent sample. All referred pathogenic variants could be targeted except one located in a repetitive genomic region. Mean time between order and validation of an assay was 14 days. Mean result reporting time was 6 days.

Conclusion: This genetic testing service was implemented as a routine practice with a simple development and interpretation process that could be offered for any paternally-inherited or *de novo* SGD. Thanks to a short turn-around time, an affordable price and a great robustness, this method has been widely adopted by clinicians nationwide for dominant paternally-inherited disorders, or as a first test for recessive disorders.

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P01.063.C Implementing non-invasive prenatal testing for aneuploidy in a national healthcare system in Russia. Moscow experience

Olesya Sagaidak[1], *Alexandra Galaktionova*[1], *Anton Olenev*[2], *Ekaterina Kuznetsova*[1], *Madina Kaplanova*[1], *Maksim Belenikin*[1], *Ekaterina Songolova*[3], *Elena Baranova*[1,4]

[1]*LLC Evogen, Moscow, Russian Federation,* [2]*Moscow City Health Department, City clinical hospital № 24, Moscow, Russian Federation, Moscow, Russian Federation,* [3]*Moscow City Health Department, City clinical hospital № 67 named after L.A. Vorokhobova, Moscow, Russian Federation, Moscow, Russian Federation,* [4]*Federal State Budgetary Educational Institution of Further Professional Education "Russian Medical Academy of Continuous Professional Education" of the Ministry of Healthcare of the Russian Federation, Moscow, Russian Federation.*

Since March 2020, NIPT has become available for women at risk up to 1:2500 in Moscow. Objectives. To evaluate the clinical efficacy of non-invasive prenatal testing (NIPT) versus prenatal screening in the first trimester of pregnancy.

Materials. According to the results of a biochemical blood test, patients were dividedinto two groups: the high-risk group (threshold ≥ 1:100) (n = 338) and the average-risk group (threshold 1:101 - 1:2500) (n = 8567). NIPT was performed for all patients. In case of high risks evaluatedby NIPT, the results were confirmed by karyotyping in the high-risk group and groupof the average-risk patients.

Results: The results of karyotyping were available in 280 patients (82.8%) of the high-risk group. High risks of trisomies by NIPT were confirmed in 66/70 cases for trisomy 21,in 22/23 cases for trisomy 18 and in 4/4 cases for trisomy 13. A high risk of fetal aneuploidies by NIPT was identified in 29 cases in the average-risk group with karyotyping available19 (65.5%) cases: 12 cases of trisomy 21 and 3 cases of trisomy 13 were confirmed, other 4 cases were false-positives in the average-risk group (including 1 case of trisomy 21 and 3 casesof trisomy 13).

Conclusion: NIPT implementation in Moscow demonstrates high efficiency and accuracy. More data is needed to choose an implementation strategy in a national healthcare system. The work was supported by Moscow City Health Department grant.

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P01.064.D Simple and complex aneuploidy in premeiotic oocytes detected by aCGH & NGS: evidence for genetic influence and effect on fertility

Harita Ghevaria[1], *Sioban SenGupta*[1], *Roy Naja*[2], *Rabi Odia*[3], *Paul Serhal*[4], *Xavier Vinals Gonzalez*[3], *Xuhui Sun*[1], *Joy Delhanty*[1]

[1]*Preimplantation Genetics Group,University College London (UCL), London, United Kingdom,* [2]*Molecular Genetics Laboratory, Igenomix UK Ltd, London, United Kingdom,* [3]*Embryology Department, The Centre for Reproductive and Genetic Health, London, United Kingdom,* [4]*Clinical Department, The Centre For Reproductive and Genetic Health, London, United Kingdom.*

Aneuploidy is the major cause of embryonic & fetal death. Most errors arise in meiosis I/II in the adult female, strongly correlated with maternal age. Our application of array comparative genomic hybridization (aCGH) and next generation sequencing (NGS) has shown that 10-15% of oocyte aneuploidy is in fact premeiotic (PM) and present in the early embryo, leading to a risk of an aneuploid conception in adult life irrespective of age. Mosaic aneuploidy maybe present in the primordial germ cells or may arise during the extensive mitotic divisions of the oogonia. A substantial individual variation in the incidence of PM errors is likely to be related to the genetic background of the oocyte donor. NGS or aCGH was performed on 141 immature oocytes [germinal vesicles (GV) & metaphase I (MI) oocytes] and 61 mature oocytes [Metaphase II - 1st PB complexes]. Fifteen (19.2%) of 78 donors had oocytes with PM errors. Oocyte aneuploidy incidence was correlated with the reproductive histories of female partners. In categories 1 & 2 most couples have fertility issues & very similar incidence of PM errors. Groups 3, 5 & 6 from couples with no known fertility issues all have lower incidences. Two cases in Group 4 stand out, with a much higher incidence which may be related to the genetic factors responsible for the onset of breast cancer in their 30s.

Table: Classification of female partners donating oocytes

Classification of female partners based on reproductive histories	Total No. of donors	Total oocytes tested	Oocytes with premeiotic aneuploidy	No. of donors contributing oocytes with premeiotic aneuploidy	No. of oocytes with Simple (SE)/ Complex (CE) Errors
1. Female fertility status unknown	27	76	11 (14.5%)	6 donors	7(SE); 4(CE)
2.Primary/secondary female factor infertility	25	48	6 (12.5%)	4 donors	3(SE); 3(CE)
3.Oocyte preservation due to social reasons	3	23	1 (4.3%)	1 donor	1(SE)
4.Oocyte preservation due to breast cancer	2	10	5 (50%)	2 donors	1(SE); 4(CE)
5.Female carriers of structural rearrangements or monogenic disorders (non-cancer related)	17	30	2 (6.66%)	2 donors	1(SE); 1(CE)
6.Females at increased risk of developing breast/ ovarian cancer due to BRCA1/2 gene mutations	4	15	0	0	0
Total	78	202	25 (12.38%)	15 donors	13 (SE); 12 (CE)

Table: Classification of female partners donating oocytes

Grant Support - CRGH

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P01.065.A Evaluation of the Telomere Length and its Effect on the Ovarian Reserve in a Sample of Colombian Women

Johana Marin Suarez[1], *Harold Moreno-Ortiz*[2], *Lissette Otero*[3], *Stephen Matlin*[3], *Clara Inés Esteban-Pérez*[2], *Maribel Forero-Castro*[1]

[1]*Universidad Pedagogica y Tecnologica de Colombia, Tunja, Colombia,* [2]*Departamento Biogenética reproductiva. Invitro, Colombia, Bogota, Colombia,* [3]*Life Length, Madrid, Spain.*

Introduction: Studies have been carried out where they relate longevity in humans with higher fertility, and indicate that delays in the onset of menopause in longer-lived women could be due to slower cellular aging; which has led us to think about the importance of the effect of telomeric shortening in reproductive aging. In addition, it has been observed that alterations in telomere length (TL) is turn related to a decrease in ovarian reserve (OR). To evaluate telomere length and its effect on OR values in healthy women with a diagnosis of PCOS.

Methodology: 66 healthy women and 44 women with PCOS, LT was quantified in mononucleated cells using the HT-QFISH technique at the Life Length Laboratory. EB was calculated from the measurement of LT. Individual RO markers were integrated with LT and EB results. The chronological and biological age of the participants was compared as well as the reproductive status.

Results: LT in both groups was in the normal range, there were no significant differences between the LT (11.7 vs 11.87, $p = 0.33$). The mean CD was 24 years for controls and 27 years for women with PCOS. There were no significant differences between CD and BE in each group (control: 24 vs 26, $p = 0.46$, and PCOS: 27 vs 26, $p = 0.9$). OR markers were higher in women with PCOS.

Conclusion: The evaluation of TL is a tool that should be explored to evaluate the reproductive status in healthy women with PCOS.

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P01.066.B Systematic review and meta-analysis of genetic association studies of pelvic organ prolapse

John W. F. Chua[1], *Kristina Allen-Brady*[2], *Romana Cuffolo*[3], *Marianne Koch*[4], *Felice Sorrentino*[5], *Rufus Cartwright*[6,7]

[1]*Zhongshan Hospital, Fudan University, Shanghai, China,* [2]*Genetic Epidemiology, Department of Internal Medicine, University of Utah, Salt Lake City, UT, USA,* [3]*Department of Obstetrics & Gynaecology, John Radcliffe Hospital, Oxford University Hospitals NHS Trust, Oxford, United Kingdom,* [4]*Department of Obstetrics and Gynecology, Medical University of Vienna, Vienna, Austria,* [5]*Department of Medical and Surgical Sciences, Institute of Obstetrics and Gynecology, University of Foggia, Foggia, Italy,* [6]*Department of Epidemiology & Biostatistics, Imperial College London, London, United Kingdom,* [7]*Department of Urogynaecology, LNWH NHS Trust, London, United Kingdom.*

Introduction and Objective: Family and twin studies demonstrate that pelvic organ prolapse (POP) is heritable, but the genetic etiology is poorly understood. This review aimed to identify genetic loci and specific polymorphisms associated with POP, while assessing the strength, consistency, and risk of bias among reported associations.

Methods: Updating an earlier systematic review PubMed and HuGE Navigator as well as relevant conference abstracts were searched using genetic and phenotype keywords from 2015-2020. Screening and data extraction were performed in duplicate. Fixed and random effects meta-analyses were conducted using co-dominant models of inheritance. We assessed credibility of pooled associations using interim Venice criteria.

Results: We screened 504 new abstracts and included 46 published and 7 unpublished studies. In pooled analyses we found significant associations for four polymorphisms: rs2228480 at the ESR1 gene (OR 0.67 95%CI 0.46-0.98, I^2=0.0%, Venice Rating BAB), rs12589592 at the FBLN5 gene (OR 1.46 95%CI 1.11-1.82, I^2=36.3%, Venice Rating BBB), rs484389 in the PGR gene (OR 0.61 95%CI 0.39-0.96, I^2=32.4%, Venice Rating CBB), and rs1800012 at the COL1A1 gene (OR 0.80 95%CI 0.66-0.96, I^2=0.0%, Venice Rating BAB). Further credible novel variants have also been recently identified in genome wide association studies.

Conclusion: The genetic contributions to POP remain poorly understood. Several biologically plausible variants have been identified, but much work is required to establish the role of these genes in the pathogenesis of POP, or to establish a role for genetic testing in clinical practice.

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P01.067.C Preimplantation genetic testing (PGT-M) for Parkinson disease (PD) risk reduction: Expanding of applications for selecting embryos

Shachar Zuckerman[1], *Ari Zimran*[2], *Talya Eldar-Geva*[3], *Elina Farhi*[1], *Shira Shaviv*[1], *Elinor Hakam-Spector*[1], *Tama Dinur*[2], *Gheona Altarescu*[1]

[1]*Medical Genetics Institute, Shaare Zedek Medical Center, Jerusalem, Israel,* [2]*Gaucher Center, Shaare Zedek Medical Center, Jerusalem, Israel,* [3]*IVF unit, Shaare Zedek Medical Center, Jerusalem, Israel.*

PGT is practiced worldwide, allowing to prevent transmission of a growing number of genetic conditions. For recessive disorders, both wild-type and carrier embryos are transferable. The justified applications for PGT are subject to ongoing debate. Mutations in the glucocerebrosidase (GBA) gene, causing Gaucher disease (GD), have emerged as a primary risk factor for PD in both patients and carriers. There is a differential effect of mild versus severe mutations: Mild mutations increase the risk of developing PD by 2.2-fold while severe mutations increase this risk by 13.6-fold. PGT counseling was given to a thirty-two years old woman with GD, compound heterozygote of N370S (mild mutation) and 84GG (severe mutation), whose mother (an 84GG carrier) died of early-onset PD associated with severe cognitive decline. GBA sequencing of her spouse was negative. The couple needed IVF. PGT-M was performed to select for N370S carrier embryos because of the reduced risk for PD, compare to 84GG carrier embryos. Eight embryos were sampled: Four were carriers for 84GG mutation; 1 aneuploid; 1 had no result; 2 were N370S carriers and thus transferable. This case report demonstrates the expansion of PGT-M use for selecting embryos with reduced risks for late-onset conditions. These novel indications for PGT-M will increase the numbers of people who would be candidates for PGT-M. The medical and bioethical consideration of these cases should be acknowledged by the professional community and discussed with couples in genetic counseling setting. Furthermore, Guidelines for PGT usage for risk reduction of late-onset disorders should be implemented.

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P01.068.D Structural fetal defects after preimplantation genetic testing - high throughput genetic investigations

Radostina Raynova[1,2], *Petya Chaveeva*[2], *Tanya Milachich*[2], *Momchil Rizov*[2], *Tanya Timeva*[2], *Atanas Shterev*[2], *Ivanka Dimova*[2,3]

[1]*National Genetic Laboratory, Sofia, Bulgaria,* [2]*SAGBAL"Shterev", Sofia, Bulgaria,* [3]*Medical University, Sofia, Bulgaria.*

Preimplantaion genetic diagnostics (PGD) is a useful approach for reducing miscarriage and increase successful pregnancy rate in couples, carriers of balanced structural rearrangements. Robertsonian translocation (RT) is one of the most common balanced structural aberrations. Previous studies of embryos' chromosomes in RT carriers subjected to PGD have shown abnormal chromosome segregation, resulting in high levels of mosaic embryos.

Here we report a case of PGD in a couple with two previous miscarriages. Cytogenetic analysis of the partners revealed RT in the woman: karyotype 45, XX der(14;22)(q10;q10). Because of the high risk for chromosomal abnormalities in the fetus, assisted reproduction with PGT-A was recommended and performed by Next Generation Sequencing after Whole Genome Amplification using Veriseq (Illumina) protocol.

Three embryos were tested at day 5, resulting in an euploid embryo, one complex aneuploidy; and a mosaic embryo - 46/46, −15; +17, dup(5)(q31.1qter). Transfer was done with the euploid embryo, but pregnacy was not realized. Mosaic embryo was transferred afterwards and pregnancy was detected. In the first trimester severe cleft palate was detected in the fetus and CVS was performed followed by array CGH analysis. The result showed terminal duplication of 12q- 46, XN, dup(12q24.23q24.33), not detected during PGT-A. Amniocentesis was performed to exclude placental mosaicism, followed by Vista™ Chromosome Sequencing technology - 12qterminal duplication was confirmed and pregnancy was terminated.

This case demonstrates the need for careful evaluation of pregnancies in which mosaic embryos are transferred; with emphasize on the use of high- throughout whole genome techniques for copy number analysis.

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P01.069.A Embryo transfer decision dilemma in PGT-M: Dominant or recessive inheritance

***Suleyman Aktuna**, Merve Polat, Evrim Unsal, Leyla Ozer, Volkan Baltaci*

Yüksek Ihtisas University, Ankara, Turkey.

Introduction: Among the PGT-M cases with a history of affected children homozygous for a recessive mutation, transfer decision of heterozygous embryos becomes challenging in case where the mutation is associated with an additional disease with autosomal dominant inheritance. Necessity of pretreament genetic counselling for these couples and the risk of heterozygous embryo transfer due to possible penetrance differences together with the patient management will be discussed in detail.

Materials and Methods: PGT-M was performed with direct mutation and haplotype analyses.

Results: This study investigates PGT-M results of 10 couples carrying different rare genetic conditions and 10 causative genes presented in Table 1. Eventough these genes are associated with multiple OMIM phenotypes with both autosomal dominant and autosomal recessive inheritance all reported cases presented autosomal recessive inheritance pattern. The total number of embryos analysed was 60 from 2 to 14 for each patient. PGT-M analysis revealed that, 15 embryos were normal, 33 were heterozygous and 11 were mutant.

Conclusions: Homozygous normal embryos should be prioritized for transfer to exclude clinical phenotype that may ocur due to potenital penetrance and expressivity differences. Normale mbryo was not detected in 3 cases, thus embryo transfer was cancelled with patient decision. This situation underlie the need for better understanding of this phenomenon during PGT-M management to prevent reduction of reproductive chance of patients coping with such tedious treatment procedures.

Table 1: Phenotype, gene, variant and variant classification details of all 10 PGT-M cases.

Phenotype	Gene	Variant classification
Cholestasis, progressive familial intrahepatic 3	ABCB4	Class 3
Hypophosphatasia, childhood Hypophosphatasia, infantile	ALPL	Class 2
Epidermolysis bullosa dystrophica	COL7A1	Class 2
Cardiomyopathy, dilated, with woolly hair and keratoderma Epidermolysis bullosa, lethal acantholytic Skin fragility-woolly hair syndrome	DSP	Class 2
Ellis-van Creveld syndrome	EVC2	Class 1
Fumarase deficiency	FH	Class 1
Spondylocarpotarsal synostosis syndrome	FLNB	Class 2
Glanzmann thrombasthenia	ITGA2B	Class 3
Mucopolysaccharidosis type IIIB	NAGLU	Class 3
Hypotonia, infantile, with psychomotor retardation and characteristic facies 1	NALCN	Class 3

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P01.071.C The shift towards rare: Increased frequency of rare PGT-M cases

***Leyla Ozer**, Evrim Unsal, Merve Polat, Suleyman Aktuna, Volkan Baltaci*

Yuksek Ihtisas University, Ankara, Turkey.

Introduction: Initial step of PGT-M is the detection of causative mutation. This has been the limiting factor for patients with rare disorders due to complex phenotype or genetic heterogeneity. Improvements in genomic technologies such as WES has become an effective tool for delineating the mutations to enable PGT-M for rare disorders. This study summarizes the variability and distribution of genes from our 1085 PGT-M cases during 2014-2021 highlights the shift towards rare disorders.

Materials and Methods: PGT-M was performed using STR based protocol for direct mutation and haplotype analysis.

Results: Retrospective data show that PGT-M was frequently performed for 6 genetic loci (Table 1). Table 2 compares the frequency 6 common disorders at different time intervals.

Conclusions: Results show that PGT-M was performed for 94 different genes during 2014-2017 while it reaches 323 in 2021. The highlight of the results was decrease in the freqeuncy for total number of common disorder cases (50,2% to 42,4%). Furthermore resuts reveals that for 90% of genes only 1-4 PGT-M cases were refererred which underlines the shift towards rare disorders with the extended utilization of WES and increase in novel gene variants.

Table 1

Distribution of cases and gene

Number of cases	Total gene number
1 case	192 (59,5%)
2-4 cases	99 (30,6%)
5-15 cases	26 (8,1%)
over 15 case (common disorders) (HBB, SMN1, DMD, CFTR, HLA, FMR1)	6 (1,8%)
Total	323

Table 2

	PGT-M cases perfomed during 2014-2017	PGT-M cases perfomed during 2017-2021
PGT-M number for common disorders	**123 (50.2%)**	**356 (42.4%)**
Total number of PGT-M	245	840

L. Ozer: None. **E. Unsal:** None. **M. Polat:** None. **S. Aktuna:** None. **V. Baltaci:** None.

P01.072.D Placental tissue co-expression networks across Russians and Yakuts identify key genes and pathways for preeclampsia

***Ekaterina Trifonova**, Anastasia Babovskaya, Aleksei Zarubin, Anton Markov, Vadim Stepanov*

Research Institute of Medical Genetics, Tomsk National Research Medical Center of the Russian Academy of Sciences, Tomsk, Russian Federation.

Population transcriptomics is a promising approach for finding loci of susceptibility to diseases which frequency depends from the ethnicity. Here we investigate the population transcriptomics in a preeclampsia (PE) model using WGCNA. The study included whole-genome expression samples from 21 Russian (Indo-Europeans population) and 23 Yakut (Mongoloids population) women with PE and physiological pregnancy. We found 10

clusters for Russian containing 7968 genes associated with PE. The main categories and pathways in these clusters are processes of the cytokine signaling (FDR = 0,005). For Yakut we found 9 clusters yielded 7966 PE-genes. The main GO-categories of these genes are amino-acid metabolism (FDR = 0,003), regulation of receptor interactions (FD = 0,047) and PI3K-Akt signaling pathway (FDR = 0,023). According to the MCC analyze of CytoHubba there were 10 hub genes with rank = 1 for Russians (*CUL1, ANAPC11, LNX1, CDC20, UBE2L6, FBXO9, KLHL13, UBA3, KCTD7, RNF111*) and Yakut (*KLHL3, FB11PSXL, ASB2, LRRC41, LMO7, RNF7, SKP2, FBXO2*). These genes are responsible for the regulation of the ubiquitin-ligase complex. In the second step we pick-out 1701 common genes for both populations. Most functionally active (rank = 1-2) remained the genes of the ubiquitin-ligase family and key processes belong to the pathways of interferon signaling (FDR = 0,004). Thus, we found population-specific pathways of PE: these are the processes of immune response for Indo-Europeans and the processes of ligand-receptor interaction for Mongoloids. In addition, we have identified common PE-genes for populations which are associated with the processes of interferon activity and operation the ubiquitin-ligase complex. Supported by RFBR №18-44-700007, №18-29-1304.

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P01.073.A Prenatal phenotype of PNKP-related primary microcephaly associated with variants in the FHA domain

Ilona Krey[1], *Sonja Neuser*[1], *Annemarie Schwan*[2], *Tobias Bartolomaeus*[1], *Jan Henje Döring*[3], *Steffen Syrbe*[3], *Margit Plassmann*[4], *Stefan Rohde*[5], *Christian Roth*[6], *Helga Rehder*[7,8], *Rami Abou Jamra*[1], *Diana Le Duc*[1], *Susanna Schubert*[1], *Katharina Schoner*[8], *Bernt Popp*[1]

[1]*Institute of Human Genetics, University of Leipzig Medical Center, Leipzig, Germany,* [2]*MVZ Dr. Eberhard & Partner Dortmund, Dortmund, Germany,* [3]*Department of Pediatrics, Hospital for Children and Adolescents, Heidelberg University Hospital, Heidelberg, Germany,* [4]*Praxis für Pränatalmedizin, Dortmund, Germany,* [5]*Department of Radiology and Neuroradiology, Klinikum Dortmund, Dortmund, Germany,* [6]*Department for Pediatric Radiology, University of Leipzig Medical Center, Leipzig, Germany,* [7]*Institute of Medical Genetics, Medical University Vienna, Wien, Austria,* [8]*Institute of Pathology, Philipps-University Marburg, Marburg, Germany.*

Biallelic polynucleotide kinase 3′-phosphatase (PNKP) variants cause different disorders ranging from neurodevelopmental disorder with microcephaly/seizures to adult-onset Charcot-Marie-Tooth disease. To date, only postnatal descriptions have been described.The index was a male fetus with prenatally diagnosed micro- and brachycephaly, brain malformations and microretrognathia at 13th gestational week. A recessive disorder was suspected because of previous termination of pregnancy (TOP) for similar abnormalities in a sister fetus. Prenatal exome sequencing using DNA derived from amniocentesis and from both unrelated parents identified compound-heterozygosity for the variants c.498G>A, p.[(=),0?] and c.302C>T, p.(Pro101Leu). Segregation analysis confirmed both variants in the affected previous fetus. Syndromic oriented fetopathology after TOP of the male index fetus revealed micrencephaly with pronounced hypoplastic frontal lobes, shortened occipital lobes, missing temporo-parietal lobulation and hypoplastic cerebellum. To characterize aberrant splicing of c.498G>A, we performed RT-PCR analysis on RNA from fetal muscle and a paternal PAXgene sample. This confirmed skipping of exon 4 affecting the FHA- and phosphatase-domains of *PNKP* (p.Leu67_Lys166del). To compare prenatal/postnatal phenotypes, we retrospectively investigated two unrelated male individuals diagnosed with biallelic *PNKP*-variants. Both carry the same splice-donor variant c.1029+2T>C and a second missense variant in the FHA-domain (c.311T>C, p.(Leu104Pro) and c.151G>C, p.(Val51Leu), respectively). RNAseq showed complex splicing events for c.1029+2T>C and c.151G>C. Computational modelling revealed significant clustering of the missense variants in the FHA-domain.Here, we present the first prenatally diagnosed *PNKP*-related primary microcephaly-/encephaly associated with variants affecting the N-terminal FHA-domain. Our detailed clinical and mutational characterisation extend the continuum of *PNKP*-manifestation to the prenatal stage.

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P01.074.B Pregnancy loss and Exome sequencing analysis (WES)

Aicha Boughalem[1], *Detlef TROST*[1], *Constance Wells*[2], *Audrey Lamouroux*[2], *Viorica Ciorna-Monferrato*[3], *Amandine Diakiese*[4], *Pascale Kleinfinger*[4], *Laurence Lohmann*[4], *Armelle Luscan*[4], *Mylene Valduga*[4], *Jean-Marc Costa*[1], *Patricia Blanchet*[2]

[1]*Laboratoire CERBA, Saint Ouen l'Aumône, France,* [2]*Département de Génétique Médicale, Centre de Référence « Anomalies du Développement et Syndromes Malformatifs », CHU Hôpital Arnaud de Villeneuve, Montpellier, France,* [3]*Génétique Médicale et Oncogénétique, Hôpital Femme Mère Enfant, Metz-Thionville, France,* [4]*CERBA, Saint Ouen l'Aumône, France.*

Background: Genetic abnormalities are known to cause pregnancy loss. In our cohort we evaluated the usefulness of exome sequencing (WES) in identifying the genetic etiology for pregnancy loss.

Methods: A cohort of 68 samples from products of conception or from fetal tissue, with normal karyotype and absence of pathogenic copy-number variants were selected for WES. WES data were analyzed based on the prenatally observed ultrasound findings and results of fetal autopsy.

Results: WES detected 12 pathogenic variants, 3 likely pathogenic variants, and 3 variants of uncertain significance (VUS) from this cohort. The Diagnostic yield for pathogenic and likely pathogenic variants was 22% and reached 26% with the inclusion of VUS. In 9 fetuses (50% of cases) the diagnoses followed an autosomal recessive inheritance pattern in nonconsanguineous couples (2 homozygous variants, 6 compound heterozygous variants and one variant with X-linked recessive inheritance) and in 9 cases the identified pathogenic variant occurred "de novo". Affected genes included those associated with ciliopathic human genetic disorder, multisystem abnormalities, neurodevelopmental disorders, cardiac anomalies, renal diseases, skeletal dysplasia and metabolic disorders.

Conclusion: These results supported the clinical utility of WES for detecting the monogenic etiology of pregnancy loss. The identification of disease-associated variants provided information for follow-up genetic counseling of recurrence risk and management of subsequent pregnancies. Our cohort study illustrates how high-resolution obstetric scan, detailed observation of fetal features and application of exome sequencing; contribute to elucidate the etiology of pregnancy loss. Keywords: Exome sequencing (WES); foetopathology; pregnancy loss, medical termination of pregnancy.

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P01.076.D Prenatal exome sequencing unravels unique co-occurrence of Congenital Idiopathic arterial calcification and congenital Gaucher disease: A potential pitfall for counseling

Sara Hisham El-Dessouky[1], *Mohamed Elhodiby*[2], *Ghada M. H. Abdel-Salam*[3]

[1]*Prenatal Diagnosis & Fetal Medicine Department, Human Genetics and Genome Research Division, National Research Centre, Cairo, Egypt,* [2]*Department of Obstetrics and Gynecology, Faculty of Medicine, M.U.S.T. University, Cairo, Egypt,* [3]*Clinical Genetics Department, Human Genetics and Genome Research Division, National Research Centre, Cairo, Egypt.*

Introduction: The presence of two or more genetic disorders represents a challenge for clinical and molecular diagnosis. If one condition remains undiagnosed, the risk of recurrence may not be appropriately assessed. In this study, we present a complex fetal phenotype that occurred in two successive pregnancies.

Materials and Methods: Prenatal ultrasound at 25 weeks revealed the constellation of polyhydramnios, hydrops fetalis, hepatosplenomegaly, arthrogryposis, microcephaly, and developmental brain anomalies in the form of intracranial calcifications, ventriculomegaly and cerebellar hypoplasia. Additionally, extensive vascular and cardiac calcification in the form of diffuse, echogenic cardiac outflow tracts, generalized hyperechogenicity in tricuspid, pulmonary valves, diffuse intrahepatic, renal and placental calcifications were observed. The fetus suffered intrauterine demise at 30 weeks gestation. Autopsy confirmed the prenatal findings. Prenatal exome sequencing (PWES), karyotyping and microarray were arranged from the fetal blood.

Results: PWES identified two novel homozygous missense variants in both the *GBA* gene (c.170G>A, p.Cys57Tyr) and the *ABCC6* (c.3381G>A, p.Met1127 Ile) gene pointing to the co-occurrence of congenital idiopathic arterial calcification and congenital Gaucher disease. All identified variants were confirmed by Sanger sequencing and validated by parental testing

Conclusion: This is the first report of congenital idiopathic arterial calcification caused by homozygous missense pathogenic variant in *ABCC6* gene and in combination with Gaucher syndrome. We believe that comprehensive phenotyping is essential for improving the diagnostic performance of PWES. Additionally our study emphasizes that unexplained phenotypes may result from the occurrence of pathogenic variants of two or more genetic disorders in the same patient.

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P01.077.A Exome sequencing in prenatal diagnosis: results from 88 cases

Emmanuelle Ranza[1], *Xavier Blanc*[1], *Federico Santoni*[1], *Frédéric Guerry*[1], *Bernard Conrad*[2], *Isabelle Eperon*[3], *Cécile Tissot*[4], *Cécile Deluen*[2], *Tanguy Araud*[2], *Loïc Baerlocher*[2], *Yasmine Sayegh Martin*[5], *Anne-Claude Müller-Brochut*[6], *Christian Bisch*[3], *Joel Fluss*[7], *Jean-Marie Pellegrinelli*[7], *Marta Carrasco*[8], *Thibaud Quibel*[9], *Sylvie Lacroix*[9], *Philippe Extermann*[3], *Graziano Pescia*[10], *Romaine Robyr Susini*[9], *Stylianos E. Antonarakis*[1]

[1]*Medigenome, Swiss Institute of Genomic Medicine, Geneva, Switzerland,* [2]*Genesupport, Geneva, Switzerland,* [3]*Dianecho, Geneva, Switzerland,* [4]*Clinique des Grangettes, Geneva, Switzerland,* [5]*Centre d'Imagerie Jean-Violette, Geneva, Switzerland,* [6]*Centre GynEcho, Fribourg, Switzerland,* [7]*HUG, Geneva University Hospitals, Geneva, Switzerland,* [8]*GHOL, Groupement hospitalier de l'Ouest Lémanique, Nyon, Switzerland,* [9]*EchoFemme, Geneva, Switzerland,* [10]*Genesupport, Lausanne, Switzerland.*

Exome sequencing (ES) has become a standard test for undiagnosed developmental disorders, now increasingly used in prenatal diagnosis. We report here our experience with 88 consecutive prenatal cases through ES. The most common fetal signs were: increased nuchal translucency (25 %), congenital heart defect (22.7 %), skeletal malformations (22.7 %) and brain abnormalities (11.4 %). After performing a-CGH, 28 cases had solo and 60 trio ES. In 25 % of cases, we have identified a pathogenic or likely pathogenic variant (as per the ACMG guidelines) likely causative of the fetal phenotype. The diagnostic yield was 40 % for skeletal malformations, and roughly 20 % for nuchal translucency, congenital heart defect, and brain anomaly cases. In 18 of the solved cases, the pathogenic variants were SNVs, while 2 were pathogenic structural variants (SV). Furthermore, the trio ES has identified in two cases complete uniparental disomies UPD6 and UPD17, both including isodisomic segments with likely causative recessive genes. ES is a powerful and accurate method for the identification of causative variants in prenatal; trio sequencing reduces the turnaround time and likely increases the diagnostic yield. The VUS remain a diagnostic challenge and international guidelines are needed to assist in the interpretation and disclosure of the results. The discovery of novel mendelian genes and the introduction of additional laboratory and computational methods such as long-read sequencing and improvement of SV detection may further increase the diagnostic yield.

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P01.078.B Mosaic Trisomy 7: A case report of discrepancies between noninvasive screening for fetal trisomy, array CGH, cytogenetics, and fetal ultrasound

*Dorela Kroni[1], **Merita Xhetani**[2], Marina Baldi[3]*

[1]StemGen Partners Ltd, University of Medicine, Immunohematology Department, Tirana, Albania, [2]University of Tirana, Faculty of Natural Sciences, Department of Molecular Biology, Tirana, Albania, [3]Genoma Laboratories, Genetic Department, Roma, Italy.

Introduction: Here we report a case of mosaic trisomy 7 diagnosed incidentally, by the prenatal screening of cfDNA (CentoNIPT®) in a31 years old pregnant women resulted inconclusive for trisomy 21, 18, and 13 at 15 weeks of gestation. Array CGH was performed at 18 weeks of gestation. Male karyotype with mosaic chromosome 7 trisomy 7p22. 3q36. 3 was reported. Ultrasound reports on fetal growth and development were normal. Follow-up with cytogenetics karyotyping at 21 weeks of gestation was performed, which revealed a result of apparently normal male karyotype 46, XY in cultured amniocytes. Simultaneously DNA testing of both parents and fetal cultured amniocytes was performed by PCR and UPD was excluded. Two contradictions have led us to publish this case: first, confirmation of reported limitation of NIPT in the detection of mosaicism, a trisomy 7 would have been reported, taking into consideration that the analysis is performed on cfDNA with placental and fetal origin, the result of mosaic trisomy 7 might have been related to a placental mosaicism situation, which was confirmed from Array CGH analyses. Second, cytogenetics on cultured amniocytes confirmed that fetal cells don't have trisomy 7 but either array CGH nor cytogenetics were diagnostic and confirmed the origin of the mosaic trisomy 7.

Conclusion: We suggest the necessity of a full panel NIPT instead of a narrow panel NIPT for better time management of pregnancy and in mosaic trisomy 7 where UPD is excluded.

D. Kroni: None. **M. Xhetani:** None. **M. Baldi:** None.

P01.079.C The contribution of chromosomal abnormalities in the formation of sporadic and recurrent early reproductive losses

***Iryna Tkach**[1], Nataliya Huleyuk[1], Danuta Zastavna[1], Thomas Liehr[2], Ewa Ciszkowicz[3]*

[1]Institute of Hereditary Pathology, NAMS of Ukraine, Lviv, Ukraine, [2]Jena University Hospital, Friedrich Schiller University, Institute of Human Genetics, Jena, Germany, [3]Department of Biotechnology and Bioinformatics, Faculty of Chemistry, Rzeszow University of Technology, Rzeszow, Poland.

Background: Spontaneous abortion occurs in 15-20% of clinically recognized pregnancy. The karyotype of a spontaneously aborted of products of conception (POC) provides valuable clinical information for the couple as well for basic research becouse provide important information for the recurrence risk of cytogenetic abnormalities in subsequent pregnancies **Aim**. The aim of the present study was to investigate the contribution of numerical chromosomal imbalances in products of conception from sporadic pregnancy loss (SPL) and recurrent pregnancy loss (RPL).

Methods: Banding cytogenetic and interphase mFISH with the probe panel for chromosomes 13, 14, 15, 16, 17, 18, 21, 22, X and Y.

Results: Cytogenetic studies of 1720 spontaneously aborted fetuses were performed. The results were stratified in 2 groups according to anamnesis: Group I (POC of first pregnancy and POC from lost pregnancy, with children in anamnesis) - 1199 samples and Group II (POC from RPL) - 521 samples. The contribution of chromosomal abnormalities was higher in the genesis of SPL (40.1%) compared to RPL (39.2%). Among most often diagnosed chromosomal change was triploidy - 24.53 % in SPL vs 24.51% in RPL, monosomy X - 21.62% vs 17.16% and trisomy 16 - 20.99% vs 26.96%.

Conclusions. Consequently, detected of chromosome aneuploidies in samples from products of conception is a key part of the investigations of reproductive failure in humans.

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P01.080.D Genetic testing of miscarriages using a QF-PCR /aCGH/ MLPA strategy: five years experiences from North-Eastern Slovenia

***alenka erjavec škerget**, boris zagradišnik, andreja zagorac, špela stangler herodež, nadja kokalj vokač*

University Clinical Centre Maribor, maribor, Slovenia.

Introduction: Traditional testing of miscarriage samples involved culture of tissue followed by G-banded chromosome analysis; this approach has a high failure rate, is labour intensive and has a resolution of around 8-10 Mb. To improve diagnostic yield and efficiency we have updated our testing strategy in 2016. Since then we use more comprehensive strategy: QF-PCR assay for all samples, followed by array CGH or MLPA, depends on a gestational age of the embrional material. Here we describe the results from the last 5 years of our strategy.

Methods: Fetal tissue samples and maternal samples were tested using QF-PCR for the detection of aneuploidy for chromosomes 13, 15, 16, 18, 21, 22, X and Y. Confirmed fetal samples without aneuplody of tested chromosomes and less than 15 weeks of gestational age were then tested by MLPA while fetal samples older than 15 weaks were analyzed by aCGH.

Results: From 335 analysed samples, 266 samples were confirmed as fetal material (78,57%). In those QF-PCR analysis identified aneuploidy/triploidy in 21.42%. MLPA detected subtelomeric imbalances in a further 12.79% (11 out of 86) while aCGH analysis detected imbalance in 4.87% (6 out of 123) of samples. All detected imbalances by aCGH were submicroscopic (in range 0.19-2.6Mb) and 2 out of 6 (33.33%) were classified as causative for the spontaneous misscariage.

Conclusions: This efficient QF-PCR/aCGH/MLPA strategy has a lower failure rate and higher diagnostic yield than karyotype. It is therefore an efficient and cost-effective diagnostic testing strategy for misscarriage products.

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P01.081.A Deciphering the genetic cause of recurrent and sporadic pregnancy loss

***Rick Essers**[1,2], Ganesh Acharya[3], Salwan Al-Nasiry[2], H G. Brunner[2,4], S. P. Deligiannis[5], E. A. Fonova[6], Alexander Hoischen[4], Ants Kurg[7], Igor N. Lebedev[6], Merryn V. E. Macville[1,2], T. V. Nikitina[6], Andres Salumets[3,5], E. A. Sazhenova[6], Servi J. C. Stevens[1,2], E. N. Tolmacheva[6], Masoud Zamani Esteki[1,2]*

[1]GROW School for Oncology and Developmental Biology, Department of Genetics and Cell Biology, Maastricht, Netherlands, [2]Maastricht University Medical Center MUMC+, Department of Clinical Genetics, Maastricht, Netherlands, [3]Karolinska Institutet and Department of Women's Health- Karolinska- University Hospital,

Division of Obstetrics and Gynecology- Department of Clinical Science- Intervention & Technology CLINTEC, Stockholm, Sweden, [4]Radboud University Medical Center, Department of Human Genetics, Nijmegen, Netherlands, [5]Institute of Clinical Medicine-University of Tartu, Department of Obstetrics and Gynecology, Tartu, Estonia, [6]Tomsk National Research Medical Center, Research Institute of Medical Genetics, Tomsk, Russian Federation, [7]Institute of Molecular and Cell Biology- University of Tartu, Department of Biotechnology, Tartu, Estonia.

Introduction: Spontaneous abortion (SA) occurs in 10-15% of clinically recognized pregnancies and recurrent pregnancy loss (RPL) in 1-3%. One potential cause for pregnancy loss is uniparental disomy (UPD). UPD can lead to imprinting disorders characterized by features affecting growth and development in liveborn offspring. This study aims to investigate the prevalence and effect of (mosaic) *de novo* genomic aberrations in RPL and SA.

Materials and Methods: We recruited 32 families with pregnancy loss (n = 16 RPL cohort, n = 16 SA cohort) with normal karyotyping results in both parents and the fetus. DNA was isolated from blood of both parents and placental tissues from the miscarried products of conception. The tissues originate from the extraembryonic mesoderm (EM) and the chorionic villi (CV). We performed SNP-genotyping (Illumina's Global-Screening Array-24 v2.0 BeadChips) and applied haplarithmisis to delineate allelic architecture of fetal tissues.

Results: We analyzed 132 DNA samples (n = 32 families). Within the RPL cohort, we found aberrations in 6/16 families including: mosaic genome-wide hexaploidy and tetraploidy, non-mosaic genome-wide hetero UPD, mosaic UPD of chromosomes 14, 16, 6, and 5 in different families. Within the SA group, we found aberrations in 2/16 families including: mosaic UPD of chromosome 7, segmental UPD of chromosome 5. All UPDs were maternally inherited.

Conclusions: The prevalence of maternal UPD was 20.6% in fetal tissues. These findings could lead to a better understanding of causative factors for SA and RPL and the need for SNP-based non-invasive prenatal testing. **Grants**: EVA (KP111513), MUMC+; Horizon 2020 innovation (ERIN) (EU952516), European Commission; RFBR (20-315-90111)

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P01.082.B Regions of homozygosity in prenatal investigation: to sequence or not to sequence?

Andreea C. Tutulan-Cunita[1], *Luiza Dimos*[1], *Anca G. Pavel*[1], *Florina M. Nedelea*[1,2,3], *Azdasher Naszan*[4], *Danae Stambouli*[1]

[1]*Cytogenomic Medical Laboratory, Bucharest, Romania,* [2]*Filantropia Clinical Hospital, Bucharest, Romania,* [3]*Carol Davila University of Medicine, Bucharest, Romania,* [4]*CF2 Clinical Hospital, Bucharest, Romania.*

Introduction: Many severe genetic disorders cannot be identified prenatally by cytogenomic analyses and, often, the parents are unaware of their carrier status. ACMG recommendations for prenatal diagnosis state that WES may be considered when standard CMA and karyotype analysis did not provide a diagnosis. We present the case of a fetus of consanguineous parents with ultrasound anomalies and normal CMA result, where WES uncovered two distinct pathogenic variants.

Materials and methods: The patient had two consecutive pregnancies with fetuses with short, bent femurs. DNA was isolated from amniotic fluid. CMA and WES were done on Affymetrix 750K and on Ion Torrent S5 platform (Thermo Fisher Scientific), respectively, while Sanger sequencing used BigDye Terminator kit.

Results: Following a normal CMA result, WES was recommended. Two novel homozygous variants were found in *CRTAP* gene (c.793 +1G>T) and *GNS* (c.811A>G, p.Arg271Gly) and classified as pathogenic and likely pathogenic, respectively, according to ACMG criteria. *CRTAP* gene is associated with osteogenesis imperfecta type VII, while *GNS* – with mucopolysaccharidosis type III D. Sanger analysis confirmed that parents are carriers. Revising CMA, long runs of homozygosity (ROH), including these two genes, were found. Thus, an ultrasound anomaly led to the serendipitous discovery of the second pathogenic mutation, which otherwise would have been missed according to prenatal checks.

Conclusion: While this finding will allow the selection of the appropriate management of the future pregnancies, it invites to further discussion about when to proceed to WES in cases of normal CMA with long ROHs, regardless ultrasound results.

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P01.083.C Retrospective study of cartilage-hair hypoplasia carrier screening cases reveal frequent insertions in the promoter region of the RMRP gene

Ezen Choo, ***Trevor Smart****, Nina Sanapareddy, Irina Rakova, J. Dianne Keen-Kim*

Natera, Inc, San Carlos, CA, USA.

Introduction: Mutations in RMRP, a nuclear encoded, intronless gene, can lead to the autosomal recessive, multi-systemic disease cartilage-hair hypoplasia (CHH). Patient studies have revealed mutations in both the promoter and the transcribed region.

Materials and Methods: We retrospectively studied 51,599 healthy individuals tested with a 137 or 274 gene carrier screening panel. Samples were tested by next-generation sequencing with orthogonal confirmation by Sanger sequencing when Pathogenic or Likely Pathogenic variants were identified. Polymorphisms and Benign/ Likely Benign variants were not included in the analysis. We assessed the occurrence of alteration types and distribution across the gene.

Results: We identified 397 unique RMRP alterations, of which 20% (n = 78) were insertions. The distribution of insertions across the gene included 43 within the promoter, 8 involving the transcription initiation site, and 27 within the transcribed region. Unique insertions affecting the promoter and start (n = 51) were more prevalent and involved larger insertions (base pairs, mean = 14.4, range:1-64) than those in the transcribed region (n = 27, mean = 2.5, range:1-17).

Conclusions: Given that RNA polymerase III promoters are usually very short, it was surprising to find larger and more diverse alterations in the promoter than in the rest of the gene in our carrier population. Up to 30bp promoter insertions have been reported in CHH patients. We identified 3 alterations >30bp in the promoter region. The predicted functional significance is promoter inefficiency and reduced transcription; however, the clinical significance and risk to patient offspring is to be determined.

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Sanapareddy: A. Employment (full or part-time); Significant; Natera, Inc.. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Natera, Inc. **I. Rakova:** A. Employment (full or part-time); Significant; Natera, Inc.. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Natera, Inc. **J. Keen-Kim:** A. Employment (full or part-time); Significant; Natera, Inc.. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Natera, Inc..

P01.088.D Russian women's preferences about invasive prenatal testing

Elena Baranova[1], *Elizaveta Zaiaeva*[1], *Gaukhar Zobkova*[2], *Lyudmila Zhuchenko*[1], *Svetlana Shelykalina*[3], *Vera Izhevskaya*[4]

[1]*Federal State Budgetary Educational Institution of Further Professional Education "Russian Medical Academy of Continuous Professional Education" of the Ministry of Healthcare of the Russian Federation, Moscow, Russian Federation,* [2]*LLC Evogen, Moscow, Russian Federation,* [3]*National Research Medical University named after N.I. Pirogov of the Ministry of Health of Russia, Moscow, Russian Federation,* [4]*Research Centre for Medical Genetics, Moscow, Russian Federation.*

Introduction: In early prenatal screening, regardless of the technology used, confirmation of the fetal chromosomal abnormality (CA) is required by invasive prenatal diagnosis. Russian women's preferences about invasive prenatal testing (IPT) are poorly understood. The aim of study was to find out the readiness of pregnant women to undergo IPT to diagnose fetal CA and terminate pregnancy if pathology is detected. The material of the research: the results of a survey of 800 pregnant women from 16 regions in Russia.

Results: It was shown that 451 (56.3%) women are ready to undergo IPT with a high risk of fetal CA, 39 (4.8%) - will not do the procedure, 281 (35.1%) were undecided, 29 (3.6%) did not answer the question. Women who agreed to carry out an IPT, compared with those who decided not to carry out an IPT, more often noted the importance of obtaining the most complete and early information. Among the respondents, only a quarter of women, 209 (26.1%) are ready to terminate a pregnancy in the case of identified fetal CA, 84 (10.5%) will not terminate a pregnancy, 474 (59.2%) have not decided, 33 (4.1%) did not answer the question. Women who made the decision to terminate pregnancy in the case of identified fetal CA were older, had more children, among them there were more women with higher education.

Conclusions: The main preferences of pregnant women with regard to undergoing invasive prenatal testing and termination of pregnancy in case of detection of a chromosomal abnormality were revealed.

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P01.089.A A rare case of fetal skeletal dysplasia: TAR syndrome

Sofia Pérez[1], *Bruno Carrilho*[2], *Ana Bernardo*[2], *Ana Teresa Martins*[2], *Álvaro Cohen*[2], *Inês Carvalho*[1]

[1]*Genetic Service, Pediatric Department, Hospital de Dona Estefânia, Centro Hospitalar Universitário de Lisboa Central, EPE, Lisboa, Portugal,* [2]*Prenatal Diagnostic Center, Maternidade Alfredo da Costa, Centro Hospitalar Universitário de Lisboa Central, EPE, Lisboa, Portugal.*

Introduction: Thrombocytopenia Absent Radius (TAR) syndrome is characterized by bilateral absent radii and thrombocytopenia, sometimes associated with other skeletal, cardiac and genitourinary anomalies. It´s an autosomal recessive disorder and results from compound heterozygosity of *RBM8A* pathogenic variants (one null and one hypomorphic allele). Case Report:We report the case of a 32-year-old female, with an ongoing pregnancy of 16 weeks, no relevant personal or family history, referred to our clinic due to an altered fetal genetic study. In the 1st trimester ultrasound, an increased nuchal translucency was identified, without other changes. For this reason, a CVS was performed and the fetal microarray revealed an interstitial deletion in the 1q21.1 region. Bearing in mind that the CNV in question encompassed the morbid *RBM8A* gene, in the medical genetics consultation we requested the sequencing of the *RBM8A* gene, since the simultaneous presence of the 1q21.1 deletion and a mutation in the other allele of the *RBM8A* gene is associated with TAR syndrome. In the 18-week ultrasound, bilateral agenesis of the radius, ulna and humerus was identified, with hands visible bilaterally. The couple opted for medical termination of pregnancy. Posteriorly, the sequencing of the *RBM8A* gene revealed the pathogenic variant c.-21G> A, in hemizygosis, confirming the diagnosis of TAR syndrome. The parents' family study revealed a paternal origin of the 1q21.1 deletion and a maternal origin of the mutation in the *RBM8A* gene.

Conclusion: Fetal skeletal dysplasia comprises a complex group of disorders, where a multidisciplinary approach is essential for the diagnosis of rare clinical entities.

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P01.090.B Telomere length dynamics during human preimplantation development

Mikhail I. Krapivin, *Anna A. Pendina, Olga A. Efimova, Irina D. Mekina, Evgeniia M. Komarova, Andrei V. Tikhonov, Olga G. Chiryaeva, Alexander M. Gzgzyan, Igor Y. Kogan, Vladislav S. Baranov*

D.O. Ott Research Institute of Obstetrics, Gynecology and Reproductology, Saint-Petersburg, Russian Federation.

Telomeres are complex structures that cap the ends of chromosomes and help to maintain genomic integrity and stability. Since telomere shorten after each cell division, their restoration during the transition of DNA to a new generation is of crucial importance. We analyzed telomere length (TL) in metaphases from 67 human embryos, unsuitable for transfer into the uterine cavity due to abnormal ploidy or morphology. For metaphase preparations, zygotes and embryos were treated with colchicines, hypotonic solution and fixed on the glass slides. We analyzed TLs in 84 metaphases from zygotes, 14 metaphases from the embryos at the 3-5-cell stage, 12 metaphases from the embryos at the 6-12-cell stage, and 49 metaphases from the blastocysts. To avoid the influence of chromatin compaction, TLs were measured as relative values by dividing telomeric by subtelomeric fluorescence intensity. The comparison of relative TL medians between the stages of preimplantation development showed significant differences: TL increased in the period from the zygote up to the 3-5-cell stage (Mann-Whitney test, $p = 0.02$) and from the zygote to the 6-12-cell stage (Mann-Whitney test, $p < 0.0001$). Then, TL decreased from the 6-12-cell stage to the blastocyst stage (Mann-Whitney test, $p < 0.0001$). Thus, we observed the waves of TL changes during preimplantation human development, with a peak at 6-12-cell stage followed by a decrease up to the blastocyst stage. These alterations of TL may be a result of telomeric region

recombination and, thus, does not require telomerase activity which is absent until the blastocyst stage. Supported by RSF №18-75-10046.

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P01.091.C Two Rare Cases of Miscarriage: Aneuploid Triploidy

Mustafa Oguz Acar[1], *Sule Altiner*[1], *Nuket Yurur Kutlay*[1], *Ajlan Tukun*[2]

[1]*Ankara University, Ankara, Turkey,* [2]*Duzen Laboratories Group, Ankara, Turkey.*

Introduction: More than 50% of early pregnancy losses have a chromosomal abnormality. Triploidy is accounting for ~10% of all spontaneous abortions.Here, two fetuses with atypical triploid karyotype referred to medical genetic department due to miscarriage at the 12th gestational week will be presented.

Materials and Methods: Conventional cytogenetic and FISH analyses of both abort tissues were carried out according to standard procedure, following tissue culture. STR analysis was used to determine the parental origins of the tetraploid chromosomes.

Results: Karyotyping revealed, 70,XXY,+18 and 71,XXXX,+6 formations, respectively. Presence of extra chromosomes was confirmed with FISH analysis. As expected, karyotypes of both parents were normal.

Conclusions: Although triploidy is common in abortion materials, the case accompanied by tetrasomy of some chromosomes has been rarely reported in the literature. Possible mechanisms to explain these chromosomal abnormalities will be discussed in detail.

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P01.092.D Triploidy/Vanished Twin Detection: Updated clinical experience following Single Nucleotide Polymorphism-based NIPT twins validation

Val Kantor*, Kathryn Young, Wendy DiNonno*

Natera, Inc, San Carlos, CA, USA.

Introduction: Occurring in ~1-2% of clinically recognized pregnancies, triploidy carries risk for miscarriage, fetal anomalies, and maternal complications. Detection is critical for medical management. Single nucleotide polymorphism (SNP)-based NIPT was previously validated to detect additional fetal haplotypes consistent with triploidy, vanished twin, or viable twin gestation. In October of 2017 viable twin gestations were validated for SNP-based NIPT. This study determines an updated PPV for triploidy using SNP-based NIPT, following the removal of known viable twin gestations.

Methods: Retrospective outcome was obtained on 1005 cases with vanished twin/triploidy results, following a SNP-based NIPT's twin validation. Pregnancy outcomes were defined as: confirmed triploidy, suspected triploidy, confirmed vanished twin, suspected vanished twin, and normal singleton.

Results: Of 1005 cases, outcome was obtained for 786 (78.2%) cases. Including only cases of confirmed triploidy, data analysis indicates a triploidy PPV of 59/786 (7.5%). If cases of suspected triploidy are included the PPV improves to 85/786 (10.8%). The conservative PPV for vanished twin gestations was 460/786 (58.5%). When cases of suspected vanished twins were included, the PPV improved to 499/786 (63.5%).

Conclusions: With five times the number of cases as Curnow et al., this study shows an improved triploidy PPV (7.5% versus previously reported 5.3% PPV.) When triploidy is combined with vanished twin gestation, we observed an improved overall PPV (66.0% versus 47.4%). Our cohort confirms the ability of a SNP-based NIPT to detect triploidy and shows an improved PPV that can better inform post-test counseling for both fetal and maternal complications.

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P01.093.A Trisomy 8 mosaicism in the placenta: a Danish cohort study of 37 cases and a literature review

Simon H. Thomsen

Aarhus University, Aarhus, Denmark.

Objective: To evaluate the risk of fetal involvement when trisomy 8 mosaicism (T8M) is detected in chorionic villus samples (CVS).

Methods: A retrospective descriptive study of registered pregnancies in Denmark with T8M in CVS identified through a database search and a review of published cases of T8M found through a systematic literature search and inclusion of cross references. Pregnancies with T8M in CVS and no additional numerical chromosomal aberrations were included.

Results: A total of 37 Danish cases and 60 published cases were included. T8M detected in a CVS was associated with fetal involvement in 18 out of 97 pregnancies (18.6% [95%CI: 11.4-27.7]). Eight out of 70 (11.4% [95%CI: 5.1-21.3]) interpreted prenatally to be confined placental mosaicism (CPM) were subsequently found to be true fetal mosaicisms (TFM).

Conclusion: T8M detected in CVS poses a significant risk of fetal involvement, and examination of amniotic fluid (AF) and/or fetal tissue should be offered. However, a normal result of AF still has a considerable residual risk of fetal involvement. Genetic counselling at an early gestational age is essential, and follow-up ultrasonography should be performed to predict fetal involvement if possible.*Funding:* Ida Vogel is funded by a research grant from the Novo Nordic Foundation: NNF16OC0018772

S.H. Thomsen: None.

P01.094.B Mosaicism for genome-wide paternal uniparental disomy - two prenatal cases

Raquel Lemos[1], *Cíntia Ventura*[1], *Fátima Torres*[1], *Gabriela Fernandes*[1], *Isabel Durães*[1], *Áurea Pereira*[1], *Patricia Costa*[1], *Jorge Castro*[2], *Conceição Brito*[2], ***Rita Cerqueira***[1]

[1]*CGCgenetics UNILABS, Porto, Portugal,* [2]*CHVNG, Obstetrícia, Vila Nova de Gaia, Portugal.*

Introduction: Uniparental disomy (UPD) is the abnormal situation in which both members of a chromosome pair are inherited from one parent, and the other parent's chromosome for that pair is

missing. UPD of the whole genome is not consistent with life but mosaic genome-wide UPD might be compatible with life. We present 2 cases of prenatal diagnosis with paternal uniparental isodisomy (GWpUPD) after contradictory QF-PCR and fetal karyotype results. The fetuses died and the placentas had hydatidiform moles criteria.

Methods: Cytogenetic analysis was performed on 20 metaphases, stained with GTG bands, from three independent cultures. The most frequent aneuploidies by QF-PCR test was performed after DNA extraction from the fetus sample with STR markers specific for chromosomes 13, 18, 21, X and Y. Chromosomal microarray study (aCGH) was performed, after DNA extraction from the fetus sample, using the Affymetrix Cytoscan 750K platform. Results In both cases, the result of QF-PCR test revealed a profile compatible with triploidy discrepant from the karyotype that revealed a normal female chromosomal constitution. The aCGH on the Affymetrix Cytoscan platform allowed the confirmation of the mosaicism for uniparental disomy for all chromosomes. These results together are compatible with GWpUPD.

Conclusions: The articulation between the different techniques was essential for the distinction between triploidy, mosaic for GWpUPD and a normal result. A prenatal case with a normal fibroblasts karyotype and a suspected hydatidiform mole without further studies could be misdiagnosed.

R. Lemos: None. **C. Ventura:** None. **F. Torres:** None. **G. Fernandes:** None. **I. Durães:** None. **Á. Pereira:** None. **P. Costa:** None. **J. Castro:** None. **C. Brito:** None. **R. Cerqueira:** None.

P01.095.C Reassessing the clinical pathogenicity of genomic variant based on family history in two prenatal cases

Núria Capdevila[1], *Neus Baena*[1], *Silvia Pina*[1], *Montserrat Pauta*[2], *Maria Segura*[3], *Antoni Borrell*[2]

[1]*Parc Taulí Sabadell, University Hospital, Sabadell, Spain,* [2]*Clinic Hospital, Barcelona, Spain,* [3]*Quantitative Genomic Medicine Laboratories, qGenomics, Esplugas del Llobregat, Spain.*

Introduction: Whole exome sequencing (WES) is a diagnostic tool in postnatal settings for individuals with a suspected genetic condition. Recently, it is increasingly used as a diagnostic tool in prenatal settings with a diagnostic yield ranging from 15% to 35%. The aim is to evaluate the pathogenicity of two genetic variants found by WES and inherited from one parent and determinate the risk of recurrence for the couple.

Methods: WES was performed in fetal samples with ultrasound anomalies, previous prenatal CGH-array with normal result. MedExome analysis using NextSeq (Illumina). Variant and segregation studies were confirmed by Sanger sequencing.

ResultsCase 1: A 34-years-old pregnant woman was referred due to prenatal ultrasound of frontal antlers fusion, suspicion of holoprosencephaly, clubhand and facial proboscis. In the collection of family history, it is noticed that the father presents bilateral culbfeet and hypertelorism. WES detected a paternally inherited heterozygous variant c.3323G>T *GLI2* (NM_005270). **Case 2:** Prenatal ultrasound at 18 weeks detected shortening upper limbs compatible with phocomelia in a 29-years-old woman. Family history shows the father has bilateral thumb hypoplasia and abnormal electrocardiogram. WES detected paternally inherited heterozygous variant c.663+1G>A *TBX5* (NM_181486.4). After genetic counselling the pregnancies were terminated in both cases. Initially, the variants detected were not reported as a responsible of the foetal phenotype until the family history was collected.

ConclusionThe use of WES in foetuses with ultrasound defects previous an accurate compilation of family history is required to determine the pathogenicity of the variant and specific risk of recurrence.

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P01.096.D Prenatal TRIO WES sequencing for fetal structural ultrasound anomalies

Detlef TROST[1], *Aïcha Boughalem*[1], *Amandine Diakiese*[1], *Patricia Blanchet*[2], *Viorica Ciorna-Monferrato*[3], *Pascale Kleinfinger*[1], *Laurence Lohmann*[1], *Mylène Valduga*[1], *Armel Luscan*[1], *Jean Marc Costa*[1]

[1]*Laboratoires CERBA, 95310 ST OUEN L'AUMONE, France,* [2]*CHU Hôpital Arnaud de Villeneuve, 34295 Montpellier cedex 5, France,* [3]*CHR Metz-Thionville, 57085 Metz Cedex 03, France.*

The usefulness of whole exome sequencing (WES) for genetic testing in cases presenting with fetal structural ultrasound anomalies is currently evaluated. In our cohort WES was performed on 128 fetus -parents TRIOs with fetal structural anomalies in ongoing pregnancies and normal karyotype and CNV analysis. Genomic DNA was extracted from CVS or amniotic fluid samples. WES data were analyzed based on the prenatally observed ultrasound findings. Pathogenic, or likely pathogenic, single nucleotide variants were identified in 28 of 128 (35.8%) cases, all were compatible with respective fetal structural anomalies. In 15 cases the identified pathogenic variant occurred "de novo", two times an autosomal variant was found to be of maternal origin with probably incomplete penetrance. In 11 of the studied fetuses recessive disorders were detected for couples unaware of their carrier status, these were homozygous in 7 cases (with 4 consanguineous couples) and composite heterozygous in 4 cases. We correlated the diagnostic yield to the major ultrasound findings, which may help to establish high risk ultrasound findings, needing investigation beyond fetal karyotyping or CNV analyses. Prenatal WES analyses enabled us to provide pertinent genetic counseling and to offer targeted prenatal diagnosis in case of a new pregnancy in 35.8 % of our couples.

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P01.097.A Prenatal diagnosis of Wieckaer-Wolff syndrome, a distinctive phenotype of arthrogryposis multiplex congenita caused by a 'de novo' ZC4H2 partial deletion

Armelle Duquenne[1], *Charlotte Deneufbourg*[1], *Jean Marc M. Biard*[2], *Yves Sznajer*[1]

[1]*Center for Human Genetics, Cliniques universitaires Saint-Luc, UCLouvain, Brussels, Belgium,* [2]*Obstetrics, Cliniques universitaires Saint-Luc, UCLouvain, Brussels, Belgium.*

Introduction: Arthrogryposis multiplex congenita (AMC) defines phenotype when contractures are present in ≥ 2 joints. Monogenic conditions/cytogenetic CNV have been identified in a wide range of diagnosis with AMC (Hall 2017). A distinctive syndromic form was originally described postnatally in males with 'club feet', intellectual disability, ophthalmic dyspraxia and muscle atrophy (Wieckaer 1985). Intragenic deletion/point mutations in ZC4H2 gene have been identified responsible for this X-linked condition (OMIM 314580/ZARD: 'ZC4H2 associated rare diseases'). Over time, case reports and recent largest review showed that females may be affected; haploinsufficiency was noted in 3; prenatal

presentation in 6 unrelated fetuses (Frints 2019). The Belgian consortium in prenatal diagnosis (BEMAPRE) defined SNP-array as the first-tier diagnostic approach for congenital malformation (Vanakker 2013).

Method-Case Report: A pregnant woman was referred at 22 weeks to our tertiary care center for sporadic arthrogryposis, 'unusual thin left leg', 'clinodactyly', normal growth parameters in a female fetus. Ultrasound study was otherly normal. Invasive procedure (amniotic fluid puncture) was performed and SNP-array (Affymetrix CytoScan 750K) identified a heterozygous 262kbs deletion in Xq11.2. Breakpoint encompasses exon 1 of ZC4H2; no contiguous gene deletion was noted. SNP-array to parents confirmed a 'de novo' occurrence. Parents asked for termination of pregnancy. A short neck, anomaly on 4 limbs (distal fingers camptodactyly, unusual thin and amyotrophic appearance of left leg with homolateral club foot) were noted.

Conclusion: We report on the seventh fetus with Wieacker-Wolff syndrome. Cytogenetic work-up allows for precise genetic counselling. The recurrence risk is related to X-linked gonadal mosaïcism (±10%).

A. Duquenne: None. **C. Deneufbourg:** None. **J.M. Biard:** None. **Y. Sznajer:** None.

P01.098.B Impaired WNT/beta-catenin signaling pathway in the etiology of azoospermia

Dunya Aydos[1], *Sena Aydos*[2], *Yunus Yukselten*[3], *Asuman Sunguroglu*[2], *Kaan Aydos*[4]

[1]*Ankara University Stem Cell Institute, Ankara, Turkey,* [2]*Department of Medical Biology, Ankara University Faculty of Medicine, Ankara, Turkey,* [3]*Research Laboratories for Health Science, Y Gen Biotechnology Company Ltd., Ankara, Turkey,* [4]*Department of Urology, Ankara University Faculty of Medicine, Ankara, Turkey.*

Introduction: Dysregulated WNT signaling in Sertoli cells or in germ cells is associated with male infertility, in particular the Sertoli cell-only (SCO) pattern in testis. Secreted WNT ligands are important in development and maintenance of adult tissue homeostasis and are antagonized by multiple secreted inhibitors that prevent ligand-receptor interactions, such as Wnt-inhibitory factor 1 (WIF1). Understanding the association of WNT signaling components with spermatogonia proliferation/differentiation; induction of apoptosis in postmitotic germ cells could help elucidating the etiology of azoospermia. To interrelate perturbed WNT signaling control and germ cell differentiation, we profiled expression levels of canonical WNT ligand, *WNT6* and WNT inhibitors, *WNT5B* and *WIF1*.

Materials and methods: Fold changes in expression levels of *WNT5B*, *WNT6* and *WIF1* were determined by quantitative PCR in testicular samples taken with microTESE intervention in NOA cases with SCO syndrome ($n = 10$) and control cases with OA ($n = 2$).

Results: *WNT5B*, *WNT6* transcripts were found significantly upregulated (3.51 ± 1.11 and 30.23 ± 14.04 fold-change, respectively) as compared with control cases with OA ($P < 0.05$). *WIF1* gene expression levels showed a significant ($P < 0.001$) decrease (0.41 ± 0.11 fold-change) compared with OA group.

Conclusions: In NOA cases increased *WNT5B* mRNA levels suggests an association with increased nuclear β-catenin in postmitotic germ cells and defective spermatogenesis. WNT6 secreted by Sertoli cells activates WNT/β-catenin signaling in spermatogonia. Also, a decrease in WIF1 levels causes β-catenin activation. In Sertoli cells, aberrant activation of canonical WNT signaling keeps them immature, which may interrupt male fertility via progressive degeneration of seminiferous tubules.

D. Aydos: None. **S. Aydos:** None. **Y. Yukselten:** None. **A. Sunguroglu:** None. **K. Aydos:** None.

P01.099.C Hemizygous loss of Xq26.2-q26.3 including *GPC3, GPC4* and *PHF6* in a fetus with lethal complex malformations

Christa Überbacher, *Irene Mutz-Dehbalaie, Renate Lunzer, Ingrid Weber, Johannes Zschocke, Christine Fauth*

Medical University Innsbruck, Innsbruck, Austria.

Introduction: Hemizygous deletions of parts of the X-chromosome are rare and due to the nullisomy for essential genes often incompatible with life. Typically, affected males have a contiguous gene syndrome, which includes phenotypic features of different disorders. Here, we describe the prenatal and autopsy findings of a male fetus with lethal complex malformations due to a hemizygous deletion Xq26.2-q26.3.

Methods and Results: Routine first trimester screening detected several abnormalities (nuchal edema, ascites, dextroversio cordis, exomphalos, growth retardation) which prompted molecular karyotyping. A male karyotype with a 2 Mb hemizygous interstitial deletion Xq26.2-q26.3 harbouring several disease genes was found (*GPC3*, Simpson-Golabi-Behmel syndrome 1, SGBS1, MIM #312870; *GPC4*, Keipert syndrome, KPTS, MIM #301026; *PHF6*, Borjeson-Forssman-Lehmann syndrome BFLS, MIM #301900; *HPRT1*, Lesch-Nyhan syndrome, LNS #300322). The parents decided for termination of the pregnancy. Autopsy confirmed ultrasound findings and revealed additional features/malformations like hydrocephalus, hypertelorism, cleft lip and palate, bilobar lung, agenesis of the diaphragm, polysplenia, anal atresia, syndactyly of fingers and toes, and broad distal phalanges. The majority of these findings belong to the phenotypic spectrum of SGBS1 with the exception of intrauterine growth retardation. Another notable finding is the severity of the diaphragm defect: diaphragmatic hernia is frequent in SGBS1 but agenesis of the diaphragm has not been previously reported.

Conclusion: The hemizygous interstitial deletion Xq26.2-q26.3 leads to a contiguous gene syndrome with congenital malformations mostly typical of SGBS1. Nullisomy of neighbouring genes like *GPC4* and *PHF6* may modulate/aggravate the severity of the phenotype.

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P01.100.D Analysis of preimplantation human and bovine embryos with regard to *XIST* repression on the future active X

Melis Atalar Aksit[1], *Bo Yu*[2], *Bernard A. J. Roelen*[3], *Barbara Migeon*[1]

[1]*McKusick Nathans Department of Genetic Medicine, Johns Hopkins University, Baltimore, MD, USA,* [2]*Farm Animal Health, Department of Population Health Sciences, Utrecht University, Utrecht, Netherlands,* [3]*Embryology, Anatomy and Physiology, Department of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands.*

X inactivation is the means of equalizing the dosage of X chromosomal genes in male and female mammals, so that there is only one active X in each cell. The *XIST* locus (in cis) on each additional X chromosome initiates its silence, making it an inactive X. Yet, how the active X in both males and females is protected from being silenced by its own *XIST* locus is not well understood in any mammal. Previous studies of autosomal duplications suggest that gene(s) on the short arm of human chromosome 19

genetically interact with the X chromosome to repress *XIST* function on the future active human X. Here, we examine the time of transcription of the candidate genes from human chromosome 19 and its ortholog, bovine chromosome 7, using single cell RNA sequence data from preimplantation human and qRT-PCR data from bovine embryos. Our results suggest that *XIST* on the future active X is repressed in both sexes just before, or at the time that, the pluripotent factors are upregulated during the 4-8 cell stage in the human and bovine embryo, well before the late blastocyst when *XIST* is upregulated on the inactive X in females. They also narrow the list of these putative candidate human and bovine genes.

M. Atalar Aksit: None. **B. Yu:** None. **B.A.J. Roelen:** None. **B. Migeon:** None.

P01.101.A Y-chromosome abnormalities in men with reproductive failure

Mariela Hristova-Savova, *Kalina Belemezova, Yuri Batchvarov, Petya Andreeva, Daniela Savova, Maria Yunakova, Tanya Timeva, Atanas Shterev, Ivanka Dimova*

SAGBAL"D-r Shterev", Sofia, Bulgaria.

Background: The human Y chromosome harbors genes that are responsible for testis development and also for initiation and maintenance of spermatogenesis in adulthood. Male infertility can be attributed to several factors such as cryptorchidism, varicocele, endocrinological disorders, obstruction/absence of seminal pathways, infections, alcohol consumption or chemotherapy. However, genetic alterations have also emerged as one of the leading cause of male infertility. The objective of our study was to investigate the type and frequency of Y-chromosome abnormalities in male infertility.

Materials and methods: We have analyzed by cytogenetic analysis 1063 men, attending reproductive clinic. Among them, we have applied also PCR analysis for Yq AZF microdeletions in 139 men with azoospermia and 195 men with oligoasthenozoospermia.

Results: Overall, 36 out of 1063 men were detected with some Y-chromosome cytogenetic aberrations - their types and frequency are shown in the Table.

Karyotype	Number/%
47,XYY	4/0.4%
46,XY/47,XYY; 46,XY/48,XYYY; 46,XY/48,XXYY	13/1.3%
46,XYqh+	9/0.8%
46,XYqh-	9/0.8%
46,Xinv(Y)	1/0.1%

Both numerical and structural Y-chromosome aberrations were detected in 1.7% of infertile men. Correlations with clinical and laboratory parameters were performed. PCR analysis revealed AZF a,b or c Y-deletions in 9 out of 139 azoospermic (6.5%) and in 2 out of 195 oligoasthenozoospermic men (1%).

Conclusion: Y-chromosome cytogenetic and molecular-genetic aberrations were found in 4.4% of infertile men. The accurate genetic diagnosis has a great impact on decision making for clinical management of these patients, offering in the affected patients PGT-A or sperm donor.

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P02 Sensory Disorders (Eye, Ear, Pain)

P02.001.B Identification of two deletions of the cis-regulatory region of the POU3F4 gene in patients with nonsyndromic sensorineural deafness from North Ossetia, Russia

Rena A. Zinchenko[1,2], *Tatyana A. Vasilyeva*[1], *Natalya V. Balinova*[1], *Vitaly V. Kadyshev*[1], *Elena F. Mikhailidi*[3], *Nika V. Petrova*[1], *Sergey I. Kutsev*[1]

[1]*Research Center for Medical Genetics, Moscow, Russian Federation,* [2]*N.A. Semashko National Research Institute of Public Health, Moscow, Russian Federation,* [3]*Republican Children's Clinical Hospital, Vladikavkaz, Russian Federation.*

X-linked deafness 2 (DFNX2) represents the most common form of X-linked deafness (OMIM PS304500). It is characterized by cochlear incomplete partition with fistulous communication with internal auditory canal. Mixed conductive and sensorineural deafness is developing. DFNX2 is associated with small intragenic *POU3F4* mutations or chromosome rearrangements involving Xq21 chromosome region. Rearrangements comprise 50% of DFNX2 genetic causes.

Material and methods: 65 North Ossetian patients (Ironian Ossetian ethnic subgroup from the North Caucasus region of Russia) are included into the study. MLPA analysis of the Xq21 is implied.

Results: In two unrelated male patients representing sporadic cases of X-linked deafness a deletion in Xq21.1 has been identified: hg18:chrX:g.(081676507_081728494)_(081728798_081866106)del. That removes distal *cis*-regulatory region in ~920 kb upstream from the *POU3F4* gene and does not affect its coding sequence. Chromosome break points have not been determined. Size could vary from 300 bp to 200,000 bp. Earlier, two other deletions in the same area (~8 kb and ~200 kb of size) were identified in Europe.

Conclusions: Observations of similar chromosome region deletions in patients from different populations from West Europe and the North Caucasus indicate that Xq21 region with its multiple conserved noncoding sequences is a hot spot for chromosome breaks. That requires to include Xq21 deletions screening, as well as target Sanger sequencing of the *POU3F4* gene in the routine analysis of the molecular causes of inherited deafness. The research was partially supported by RSF grant №17-15-01051 and within the state task of the Ministry of education and science of Russia

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P02.002.C Genetic and environmental factors influencing sensory decays during aging in a large Italian cohort

Giorgia Girotto[1], *Massimiliano Cocca*[2], *Anna Morgan*[2], *Eulalia Catamo*[2], *Paola Tesolin*[1], *Agnese Feresin*[1], *Paolo Gasparini*[1], *Maria Pina Concas*[2]

[1]*University of Trieste-IRCCS-Burlo Garofolo, Trieste, Italy,* [2]*IRCCS-Burlo Garofolo, Trieste, Italy.*

Sensory perception changes over a lifetime and its impairment play a critical role in health and quality of life. Studies published until now have focused on single sensory impairments in elderly people while data on the significant "multisensory phenotype" (MS) are still lacking. Genetic and phenotypic data (hearing, taste and smell evaluated through sensory functions assessment) of 1152 individuals have been investigated. MS was calculated as the total number of

impaired senses. The following steps have been applied: 1) regression models to assess the association between MS and personal/lifestyle characteristics, 2) GWAS meta-analysis and 3) gene-based analysis to find genetic influences on MS. Regarding (1), male gender, ageing, and low educational level were associated with MS higher values (p-value < 0.05) while no role was recognized for smoking habit and high alcohol consumption. For GWAS analysis (2), a total of seven genes resulted in being associated with MS (p-value $< 1x10^{-6}$). In particular, *BCL7C* and *MACROD2*, both expressed in the brain and in the inner ear, have been recently associated with Lewy body dementia and neurological disorders, respectively. Finally, gene-based analysis (3) highlighted several genes implicated in sensory signalling pathways such as *LSAMP*, *PRKG1, GNG7, RYR3, PTPRN2*. Present data show that several factors- both environmental and genetic - influence concomitant sensory declines. Further investigation (GWAS replication combined with in vivo studies in animal models) are needed to confirm our results that will ultimately help to understand better the complex biological mechanisms underlying MIS and ageing.

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P02.003.D Molecular Diagnosis of *TYR* Negative Albinism Patients by Clinical Exome Sequencing

*Sezer Akyoney[1], **Ilayda Sahin**[2,3], Büşra Ünal[4], Nihat Buğra Ağaoğlu[4], Abdulbaki Mudun[5], Zeynep Parlakgüneş[6], Engin Yılmaz[7], Yasemin Alanay[2,8], Uğur Özbek[2,8], Özden Hatırnaz Ng[1,8]*

[1]Acıbadem Mehmet Ali Aydınlar University, School of Medicine, Department of Medical Biology, Istanbul, Turkey, [2]Acibadem Mehmet Ali Aydinlar University, School of Medicine, Department of Medical Genetics, Istanbul, Turkey, [3]Acıbadem Mehmet Ali Aydinlar University, Department of Medical Biotechnology, Istanbul, Turkey, [4]University of Health Sciences, Umraniye Training and Research Hospital (UEA), Department of Medical Genetics, Istanbul, Turkey, [5]Acibadem Maslak Hospital, Department of Ophthalmology, Istanbul, Turkey, [6]Yeditepe University Eye Center, Department of Ophthalmology, Istanbul, Turkey, [7]Hacettepe University, Faculty of Medicine, Department of Medical Biology, Istanbul, Turkey, [8]Acibadem University Rare Diseases and Orphan Drugs Application and Research Center (ACURARE), Istanbul, Turkey.

Introduction: Albinism is a group of rare genetic conditions inherited autosomal recessively and associated with reduced or no melanin production. Due to high clinical/genetic heterogeneity, genotype-phenotype correlation can only be carried out by genetic diagnosis that is vital especially for syndromic forms. More than 33 genes are related to albinism and these can explain the genetic background of 70-75% of all albinism cases. Among these *TYR* is the most detected gene. Here we aimed to determine the genetic background of *TYR* negative cases by clinical exome analysis.

Materials and Methods: Nine *TYR* negative cases were studied by clinical exome sequencing. Analyzes of the raw data were carried out both with ACURARE in-house pipeline and SOPHiA GENETICS software. The sequences were mapped and aligned with the GRCh38 reference genome. The detected variants were evaluated according to the ACMG guideline. Candidate variants were validated by Sanger sequencing in patients and segregation analyzes were performed in the family members that can provide samples.

Results: A homozygous variant was identified in all nine cases and eight of them were novel. The gene variants were summarized in Table1. The family members were heterozygous carriers of the same variant detected in the index case.

Conclusion: The results obtained reveal the importance of genetic diagnosis in albinism especially in syndromic forms who might need special follow-up

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P02.004.A Clinical and genetic analysis of new cases provides further characterisation of ALDH1A3-related anophthalmia/microphthalmia

***Yesim Kesim**[1], Fabiola Ceroni[1,2], Alejandra Damián[3,4], Fiona Blanco-Kelly[3,4], Carmen Ayuso[3,4], Kathy Williamson[5], Véronique Paquis[6], Dorine Bax[1], Claudine Rieubland[7], Chamlal Mostafa[8], Marta Cortón[3,4], Nicolas Chassaing[9,10], Patrick Calvas[9,10], Nicola Ragge[1,11]*

[1]Department of Biological and Medical Sciences, Faculty of Health and Life Sciences, Oxford Brookes University, Oxford, United Kingdom, [2]Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy, [3]Department of Genetics & Genomics, Instituto de Investigación Sanitaria-Fundación Jiménez Díaz University Hospital, Universidad Autónoma de Madrid (IIS-FJD, UAM), Madrid, Spain, [4]Centre for Biomedical Network Research on Rare Diseases (CIBERER), Madrid, Spain, [5]MRC Human Genetics Unit, MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, Western General Hospital, Edinburgh, United Kingdom, [6]Department of Medical Genetics, Nice Teaching Hospital, Nice, France, [7]Department of Human Genetics, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland, [8]Department of Pediatrics, Tangier Hospital, Tangier, Morocco, [9]UDEAR, Université de Toulouse,

Variants in genes associated with albinism were detected in all patients included in the study

Patient code	Gene	Transcript	Nucleotide change	Amino acid change	MAF	Inheritance	Rs code
TG18-30	*HPS1*	NM_001322482	c.612delC	p.M205Wfs*5	0,0001	Homozygous	rs281865082
TG18-31	*OCA2*	NM_001300984	c.2186T>C	p.L729P	N/A	Homozygous	Novel
TG18-46	*OCA2*	NM_001300984	c.2186T>C	p.L729P	N/A	Homozygous	Novel
TG19-01	*SLC45A2*	NM_001012509	c.400delC	p.(Pro134Glnfs*9)	N/A	Homozygous	Novel
TG19-01	*SLC45A2*	NM_001012509	c.482G>T	p.G161V	N/A	Homozygous	Novel
TG19-30	*SLC45A2*	NM_016180	c.386-1G>A	N/A	N/A	Homozygous	Novel
TG19-36	*OCA2*	NM_000275	c.2037G>C	p.(Gln319*)	0,0000915	Homozygous	rs121918169
TG19-46	*OCA2*	NM_000275	c.1648G>A	p.(Glu550Lys)	N/A	Homozygous	Novel
TG19-48	*SLC45A2*	NM_001012509	c.328G>C	p.G110R	N/A	Homozygous	Novel
TG19-54	*SLC45A2*	NM_016180	c.386-1G>A	Splice variant	N/A	Homozygous	Novel

UMR 1056 Institut National de la Santé et de la Recherche Médicale-Université Paul Sabatier, Toulouse, France, [10]Department of Medical Genetics, Purpan University Hospital, Toulouse, France, [11]Department of Clinical Genetics, West Midlands Regional Clinical Genetics Service and Birmingham Health Partners, Birmingham Women's and Children's Foundation Trust, Birmingham, United Kingdom.

Introduction: Anophthalmia and microphthalmia (AM) are a genetically heterogeneous group of disorders that can be isolated or syndromic. Biallelic *ALDH1A3* variants are responsible for 11% of recessive AM cases, mostly described in consanguineous families, and present severe bilateral AM with variable neurodevelopmental anomalies. We present six families with biallelic *ALDH1A3* variants, further characterising the associated phenotype.

Material and Methods: AM individuals from UK, France and Spain were analysed by WES, targeted gene screening and arrays.

Results: We identified 6 families: Family 1) with two brothers with bilateral anophthalmia, one present additional developmental delay, absent speech and autism with compound heterozygous *ALDH1A3* variants (c.874G>T:p.(D292Y);c.1393A>T:p.(I465F)); 2) a boy with bilateral anophthalmia, developmental and intellectual delay, seizures and autistic features with compound heterozygous variants (c.845G>C:p.(G282A);c.1459A>G:p.(R487G)); 3) a girl with bilateral microphthalmia and coloboma with compound heterozygous variants (c.847_849del;p.(G283del);c.953C>A,:p.(S318Y)); 4) a girl with bilateral anophthalmia with a homozygous missense variant (c.1144G>A:p.(G382R)); 5) a boy with bilateral microphthalmia, unilateral coloboma and cataract, with a homozygous splice variant (c.1233+2T>C) and 6) a boy with bilateral microphthalmia, iris and chorioretinal coloboma, facial dysmorphism with a homozygous missense variant (c.434C>T:p.A145V).

Conclusions: Three of the six families presented with compound heterozygous variants, highlighting this mode of inheritance in *ALDH1A3*-related disorders. Five of 6 families had a variant in the catalytic domain, supporting the importance of this domain, which is critical for substrate selectivity. Severe neurodevelopmental phenotypes were present in two individuals, and variably penetrant in family 1, supporting that this can be an important feature of the *ALDH1A3* syndrome.

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P02.006.C Further evidence of involvement of *SERPINB6* in autosomal recessive non-syndromic hearing loss

Barbara Vona[1], *Thore Schade-Mann*[1], *Petra Stöbe*[2], *Philipp Gamerdinger*[1], *Aboulfazl Rad*[1], *Marc Sturm*[2], *Hubert Löwenheim*[1], *Tobias B. Haack*[2], *Anke Tropitzsch*[1]

[1]Dept of Otolaryngology—Head & Neck Surgery, Tübingen Hearing Research Centre, University of Tübingen, Tuebingen, Germany, [2]Institute of Medical Genetics and Applied Genomics, University of Tübingen, Tübingen, Germany, Tuebingen, Germany.

Introduction: Non-syndromic hearing loss is a genetically heterogeneous sensory disorder. Autosomal recessive hearing loss is the most prevalent form of non-syndromic hearing loss with approximately 80 associated genes to date. *SERPINB6* (serpin family B member 6, also called protease inhibitor 6) was mapped to the DFNB91 locus in 2010 and causally associated with moderate-to-severe high-frequency hearing loss.

Materials and Methods: As part of our hereditary deafness study, we ascertained a child with sensorineural hearing loss and a history of parental consanguinity. DNA from the proband was subjected to genome sequencing and bioinformatics analysis. Hearing evaluation and family history were recorded.

Results: A novel homozygous nonsense *SERPINB6* variant (ENST00000335686:c.217C>T, p.(Gln76Ter)) was identified in a 24 Mb run of homozygosity. Moderate-to-severe high-frequency sensorineural hearing loss was diagnosed at approximately six years of age.

Conclusions: The limited literature about *SERPINB6* in patients with biallelic nonsense or splicing variants hints to a postlingual onset and rapidly progressive hearing loss. Progression is consistent with loss of a functional intracellular protease inhibitor. We add to the limited clinical and molecular genetics knowledge, better characterizing *SERPINB6*-associated non-syndromic hearing loss.

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P02.007.D A new cases of Axenfelt-Rieger syndrome, caused by a novel *FOXC1* mutation and 6p25 deletion

Esra Kilic

Department of Pediatric Genetics, University of Health Sciences, Ankara City Hospital, Ankara, Turkey.

Introduction: Axenfelt-Rieger syndrome (ARS) is an autosomal dominant genetic disorder characterised by ocular anterior segment disorders with systemic involvement. Neural crest origin dysgenesis of cornea, iris, anterior angle, glaucoma, oligodontia, conical incisor teeth, hypoplastic enamel, midface hypoplasia, hearing deficit, growth and development disorder, cardiac defects and intestinal malformations are clinical findings of this disorder. Mutations or deletions in *forkhead box C1* (*FOXC1*, chromosome 6p25) are responsible 25% of ARS cases, *pituitary homeobox 2* (*PITX2*, chromosome 4q25) 55% of ARS cases.

Patients, and Results: Case 1, 3-years-old boy with developmental delay, hypothroidism, iridocorneal dysgenesis, glaucoma, midface hypoplasia, thin upper lip and enamel hypoplasia on incisor teeth. Metabolic workup, hearing, abdominal ultrasonography, echocardiography and chromosomal analysis were normal. His microarray analysis revealed that 3,3Mb sized heterozygous deletion on 6p25 including *FOXC1* gene. Case 2, 6-years-old boy with short stature, bilateral megalocornea, iris coloboma, crowded teeth with hypoplastic enamel, flat nasal bridge and everted lower lip. His developmental milestones and intellectual capacity was normal. Metabolic workup, hearing, abdominal ultrasonography, echocardiography, cranial MRI, chromosomal analysis, microarray analysis and *PITX2* sequence analysis were normal. Sanger sequencing of *FOXC1* gene revealed, novel heterozygous de novo c.76dupT (p.Y26Lfs *57) mutation.

Conclusion: Here we report two new patients of ARS, one had a novel *FOXC1* mutation, and the other had 6p25 chromosomal deletion. We also observed more severe clinical phenotype in the deletion type ARS. Ocular anterior segment disorders are clinically and genetically heterogenous conditions, by demonstrating the underlying genetic cause we gave appropriate genetic counseling and follow-up.

E. Kilic: None.

P02.008.A Brittle cornea syndrome with a novel pathogenic variant of *PRDM5* gene

Ezgi Susam[1], *Nilgün Yıldırım*[2], *Sinem Kocagil*[1], *Oguz Çilingir*[1]

[1]Eskisehir Osmangazi University Medical Genetics Department, Eskisehir, Turkey, [2]Eskisehir Osmangazi University Ophthalmology Department, Eskisehir, Turkey.

Brittle cornea syndrome is a rare syndrome, characterized by extreme thinning of cornea with estimated prevalence less than 1 in 1,000,000. Biallelic pathogenic variants of *PRDM5* and *ZNF469* genes have been identified for etiology of the syndrome.Blue sclerae, corneal thinning with or without corneal rupture, myopia, early-onset keratoconus and keratoglobus are the main ophthalmological features of the disease. Although eyes are the most severely affected organs, it is a systemic disorder including hearing loss and some connective tissue manifestations like scoliosis, hyperelasticity, developmental dysplasia of the hip and rarely increased bone fractures. Here we report a 1-year-old male patient, referred to our clinic from ophthalmology department due to keratoglobus. He was born as third child of healthy first-degree cousin parents at term without any complication. At physical examination, he presented with corneal opacity on left eye, bilateral blue sclera, micrognathia and hyperelasticity. Echocardiography and hip ultrasonography were normal. Despite he had passed neonatal auditory screening, patient was consulted to ENT department again for hearing evaluation and diagnosed with moderate sensorineural type hearing loss. With these findings, brittle cornea syndrome was thought as the most accurate diagnosis and *PRDM5* gene sequencing revealed a novel c.177 +1G>A variant in homozygous state. This variant was classified as pathogenic by ACMG guidelines and segregation analysis was compatible with parents of the proband as carrier. According to his physical examination and the results of the molecular analyses we have concluded that this novel variant causes Brittle cornea syndrome and genetic counseling was given to family.

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P02.009.B Two new *BRPF1* variants associated with IDDDFP and previously unreported ocular findings

Erika Fiorentini[1], *Samuela Landini*[2], *Lucia Tiberi*[2], *Angelica Pagliazzi*[2], *Giulia Gori*[2], *Elia Dirupo*[2], *Elisa Marziali*[3], *Pinuccia Fortunato*[3], *Giacomo Bacci*[3], *Roberto Caputo*[3], *Sara Bargiacchi*[2], *Viviana Palazzo*[2], *Rosangela Artuso*[2]

[1]Medical Genetics Unit, Department of Clinical and Experimental Biomedical Sciences 'Mario Serio', University of Florence, Firenze, Italy, [2]Medical Genetics Unit, Meyer Children's University Hospital, Firenze, Italy, [3]Pediatric Ophthalmology Unit, A. Meyer Children's Hospital, Firenze, Italy.

BRPF1 gene on 3p26-p25 encodes a protein involved in epigenetic regulation, through interaction with histone H3 lysine acetyltransferase KAT6A and KAT6B of the MYST family. Recently heterozygous variants in *BRPF1* have been identified in subjects with IDDDFP (OMIM 617333), a disorder characterized by global developmental delay, intellectual disability, language delay and peculiar facial features (round face, flat profile, broad nasal root, hypertelorism, blepharophimosis, ptosis, abnormally shaped ears). Joint hypermobility, cervical spinal fusion, EEG abnormalities and epilepsy also occur. Reported ocular problems are strabismus, amblyopia and refraction errors. We found the *de novo* heterozygous variant c.330delC (p.Ile110fs) in *BRPF1* by whole exome sequencing (WES), conducted in a patient (P1) with mild intellectual disability, ptosis and typical facies. Interestingly, the patient also had a Chiari Malformation type I and a subclinical optic neuropathy, which could not be explained by variations in other genes. WES performed in a second patient (P2) showing, as well as P1, intellectual disability, round face, ptosis and strabismus revealed the heterozygous variant c.1447_1450delGTCA (p.Val4834argfsTer11) in *BRPF1*; cerebral MRI at 1 and 3 years were normal. Having detected a peculiar ocular phenotype in P1, we suggested optical coherence tomography (OCT) for P2; such exam detected bilateral subclinical optic neuropathy also in this case. To date, only a few patients with *BRPF1* mutations have been described and none was reported to have an optic neuropathy. Since subclinical optic nerve alterations can go easily undetected, our experience highlights the importance of a more detailed ophthalmologic evaluation in patients with *BRPF1* variant.

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P02.010.C Maternally inherited hemizygous 8.5 Mb Xq21.1 deletion in a male patient with choroideremia, deafness and intellectual disability found using NGS based CNV-calling approach

Ewelina Bukowska-Olech[1], *Alexander Pepler*[2], ***Aleksander Jamsheer***[1,3]

[1]Department of Medical Genetics, Poznan University of Medical Sciences, Poznan, Poland, [2]CeGaT GmbH, Tubingen, Germany, [3]Centers for Medical Genetics GENESIS, Poznan, Poland.

Background: Xq21 deletions associate with choroideremia-deafness-obesity syndrome (MIM: 303110), which main clinical features comprise choroideremia, obesity, moderate intellectual disability and hearing impairment. This contiguous gene deletion involves at least *CHM* and *POU3F4* genes known to cause choroideremia and deafness, respectively.

Materials and methods: The male patient with choroideremia, deafness and intellectual disability underwent the whole-exome sequencing (WES). Next, he was subjected to confirmation studies using quantitative PCR (qPCR) and whole genome array comparative genomic hybridisation (array CGH) (SurePrint G3 Human CGH Microarray 1 × 1M; Agilent Technologies). Besides, we performed segregation analysis applying qPCR.

Results: We identified the maternally inherited hemizygous Xq21.1-q21.32 deletion (hg38 chrX:85003913-92618940) using WES based CNV-calling approach. The variant was calculated based upon observed versus expected coverage using exome sequencing data. Next, we confirmed the presence of aberration applying qPCR and narrowed down its size through array CGH method (hg38 chrX:84662472-93174172).

Conclusion: Our patient harbours a hemizygous Xq21 deletion of a smaller size than similar CNVs reported in the medical literature thus far. Our finding supports the contribution of CNVs to choroideremia, which is an orphan disease being tested for ocular gene therapy. Besides, we have shown the clinical utility of NGS based CNV-calling approach, which effectively allowed to detect of the CNV in the exome sequencing data.

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P02.011.D Genome-wide association study identifies *RNF123* locus as associated with chronic widespread musculoskeletal pain

Md Shafiqur Rahman[1], *Bendik S Winsvold*[2], *S.O. Chavez Chavez*[3], *Sigrid Børte*[2], *Yakov A. Tsepilov*[4], *Sodbo Zh. Shapov*[4], *HUNT All-In*

Pain, Yurii Aulchenko[5], Knut Hagen[6], Egil A. Fors[6], Kristian Hveem[6], John-Anker Zwart[6], J.B.J. van Meurs[3], Maxim B. Freidin[1], Frances M.K. Williams[1]

[1]King's College London, London, United Kingdom, [2]Oslo University Hospital, Oslo, Norway, [3]Erasmus Medical Center, Rotterdam, Netherlands, [4]Novosibirsk State University, Novosibirsk, Russian Federation, [5]PolyOmica, Maastricht, Netherlands, [6]Norwegian University of Science and Technology, Trondheim, Norway.

Background and Objectives: Chronic widespread musculoskeletal pain (CWP) is a symptom of fibromyalgia and a complex trait with poorly understood pathogenesis. CWP is heritable (48-54%), but its genetic architecture is unknown and candidate gene studies have produced inconsistent results. We conducted a genome-wide association study to get insight into the genetic background of CWP.

Methods: Northern Europeans from UK Biobank comprising 6,914 cases reporting pain all over the body lasting more than 3 months and 242,929 controls were studied. Replication of three lead genome-wide significant single nucleotide polymorphisms (SNPs) was attempted in 6 independent European cohorts (N = 43,080; cases = 14,177). Genetic correlations with risk factors, tissue specificity, and colocalization were examined.

Results: Three genome-wide significant loci were identified (*rs1491985, rs10490825, rs165599*) residing within the genes *RNF123*, *ATP2C1*, and *COMT*. The *RNF123* locus was replicated (meta-analysis p = 0.0002), the *ATP2C1* locus showed suggestive association (p = 0.0227), and the *COMT* locus was not replicated. Partial genetic correlation between CWP and depressive symptoms, body mass index, age of first birth, and years of schooling were identified. Tissue specificity and colocalization analysis highlight the relevance of skeletal muscle in CWP.

Conclusions: We report a novel association of *RNF123* locus with CWP and suggest a role of *ATP2C1*, consistent with a role of calcium regulation in CWP. The association to *COMT*, one of the most studied genes in chronic pain field, was not confirmed in the replication analysis.

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P02.012.A Unexpected NGS findings in a case of Coats' disease, combination of rare variants in the *HMCN1* and *NPHP4*genes associated with other forms of retail dystrophy

***Tatiana A. Vasilyeva**, Vitaly V. Kadyshev, Nika V. Petrova, Andrew V. Marakhonov, Rena A. Zinchenko*

Research Centre for Medical Genetics, Moscow, Russian Federation.

Coats' disease (OMIM300216) is a form of retinal dystrophy which occurs due to congenital abnormality of retinal vessels. Patients, mainly young men, show unilateral retinal telangiectasia, retinal exudation and detachment. Coats' disease occurs preferentially as sporadic cases, its genetic cause is still unknown. A 17-year-old Caucasian male patient with sporadic exudative vitreoretinopathy/Coats' disease underwent complete ophthalmological examination, whole exome sequencing by NGS method was implied for searching causative genetic variants of the phenotype. Two heterozygous variants in different genomic loci associated with other forms of retinal dystrophy have been detected, a rare variant in the *HMCN1* gene c.9571C>T p.(Arg3191Cys) and a known pathogenic variant in the *NPHP4* gene c.2930C>T p.(Thr977Met). Pathogenic variants in the *HMCN1* gene are responsible for dominant age-related macular dystrophy (#603075), variants in the *NPHP4* gene cause recessive Senior-Loken syndrome 4 (#266900). Encoding proteins are involved in the regulation of integrity of blood/retina barrier at the levels of vascular endothelium and retinal pigment epithelium. The *NPHP4* gene is expressed in connecting cilium, which normally performs important function of trafficking between the external and internal segments of photoreceptors. A mechanism of functional consequences of the detected variants combination is proposed to be accumulation of several defects, violations of blood/retina barrier, as well as diminishing of the ciliary transporting potential. The consequences can aggravate each other and lead to the observed phenotype. Carried out within the state assignment of Ministry of Science and Higher Education of the Russian Federation, supported in part by RFBR grant (No.20-015-00061).

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P02.013.B Mutation analysis in frequent genes in a cohort of Russian patients with congenital glaucoma

***Andrey V. Marakhonov**[1], Alexander A. Sorokin[2], Tatyana A. Vasilyeva[1], Sofya A. Garifullina[1], Darya M. Guseva[1], Natalya A. Semenova[1], Vitaly V. Kadyshev[1], Rena A. Zinchenko[1]*

[1]Research Centre for Medical Genetics, Moscow, Russian Federation, [2]Moscow Helmholtz Research Institute of Eye Disease, Moscow, Russian Federation.

Primary juvenile glaucoma develops due to ocular hypertension with the onset either at birth or within the first few years of life. It arises due to abnormalities in the anterior chamber angle development, that obstructs aqueous outflow in the absence of systemic anomalies or other ocular malformations. According to Orphanet data, its birth prevalence in Europe is 2.2 cases per 100,000 newborns. Congenital glaucoma could be inherited in either autosomal recessive or autosomal dominant modes. The rationale of this study was to analyze mutations in frequent genes in a cohort of Russian patients with congenital glaucoma.Twenty-one Russian patients with a primary diagnosis of congenital glaucoma were included in the study. Targeted sequencing and MLPA analysis of *FOXC1*, *PITX2* (with autosomal dominant inheritance), and *CYP1B1* (with autosomal recessive inheritance) genes were performed.In eight patients, mutations in analyzed genes were detected. One patient has previously known homozygous variant in the *CYP1B1* gene, NM_000104.3:c.685G>A. Four patients have heterozygous variants in the *FOXC1* gene: two novel (NM_001453.2:c.246C>A, c.235C>T) and two previously described (c.379C>T, c.379C>T). Three more patients have heterozygous variants in the *PITX2* gene: two novel (NM_001204397.1:c.114G>T and NM_153427.2:c.408_410delTCG) and one described earlier (NM_153427.2:c.191C>T). Thirteen patients lack variants in analyzed genes.According to our study, congenital glaucoma could be associated frequently with genes that are usually linked to anterior segment dysgenesis including Axenfeld-Rieger syndrome.Carried out within the state assignment of Ministry of Science and Higher Education of the Russian Federation, supported in part by RFBR grant (No. 19-015-00122).

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P02.014.C Congenital insensitivity to pain: molecular characterization of a novel disrupting mutation in *SCN9A*

Margherita M. Marchi[1], *Ilaria D'Amato*[1], *Mirna Andelic*[1], *Erika Salvi*[1], *Daniele Cartelli*[1], *Raffaella Lombardi*[1], *Gumus Evren*[2], *Giuseppe Lauria*[1,3]

[1]*Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy,* [2]*Department of Medical Genetics, Faculty of Medicine, University of Harran, Sanliurfa, Turkey,* [3]*Department of Biomedical and Clinical Sciences "Luigi Sacco", University of Milan, Milan, Italy.*

Aim of this study is to investigate the splicing consequence of a novel intronic mutation in *SCN9A* (NM_002977.3) in a case of congenital insensitivity to pain (CIP, #243000). CIP is an extremely rare condition caused by bi-allelic inactivating mutations in *SCN9A*, encoding the sodium channel Nav1.7, responsible for firing and transmission of noxious stimuli in the peripheral nociceptors. CIP presents clinically as the insensitivity to nociceptive pain and heat, often with anosmia.We report an 8-years-old girl, from an inbred family, diagnosed with CIP, showing absence of pain sensation, diminished temperature sensation, foot burns, normal olfaction, hearing and MRI. Next-generation-sequencing of *SCN9A* revealed a substitution c.377+7T>G in the donor splice-site of intron 3, homozygous in the girl and heterozygous in her healthy mother. Total RNA from the proband, her mother and one healthy unrelated control was extracted from blood and retrotranscribed. The cDNA region spanning exon 2 to 4 was amplified by PCR, followed by the fragments dimensional analysis on agarose gel and Sanger-sequencing. Sequencing revealed in the affected girl two mis-spliced transcripts: both lacking exon 3 and presenting a non-canonical isoform of exon 4, and one showing a partial retention of intron 2. The mis-spliced transcripts are predicted to induce a reading frame shift, which prematurely stops after two (p. Lys86fs2Stop) or twelve out-of-frame aminoacids (p.Lys86fs12-Stop), resulting in the loss of NAv1.7.This study describes a novel mutation in *SCN9A* causing the Nav1.7 deficiency underlying the nociceptors dysfunction and highlights the importance of investigating intronic mutations outside the splicing consensus.

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P02.015.D Complex inheritance of retinal degeneration: At least three mutations segregate in a family with autosomal-dominant congenital stationary night blindness

Kalina Mihova[1], *Krasimir Koev*[2], *Kunka Kamenarova*[1], *Silvia Cherninkova*[3], *Radka Kaneva*[1]

[1]*Molecular Medicine Center, Department of Medical Chemistry and Biochemistry, Medical University - Sofia, Sofia, Bulgaria,* [2]*University Hospital "Tsaritsa Yoanna", Medical University - Sofia, Sofia, Bulgaria,* [3]*Department of Neurology, University Hospital "Alexandrovska", Medical University – Sofia, Sofia, Sofia, Bulgaria.*

Introduction: Congenital stationary night blindness (CSNB) comprises a group of genetically and clinically heterogeneous non-progressive retinal disorders (RD), mainly due to rod dysfunction. This study was performed to identify the genetic defect in a large family affected with autosomal-dominant (ad) CSNB.

Materials and Methods: Sixteen affected relatives of a large Gypsy pedigree segregating adRD were examined clinically by standard ophthalmological methods. Based on this, 15 patients were diagnosed as having CSNB and one presenting Retinitis pigmentosa (RP). Whole exome sequencing was performed in CSNB patients. A systematic filtering approach coupled with copy number variation (CNV) analysis was used to identify pathogenic variants, subsequently verified by Sanger sequencing and segregation analysis. Additionally, MLPA analysis was used to confirm presence of CNVs.

Results: A novel *RHO*-mutation (c.803A>G, p.Tyr268Cys) was identified in 5 patients with adCSNB. They had full vision under photopic conditions, showed no fundus abnormalities but presented night blindness with an altered scotopic ERG. A known *RHO* mutation, c.541G>A (p.Glu181Lys), was found in the patient presenting typical signs of RP. CNV and further MLPA analysis detected third, novel, *IMPDH1*-exon-17 heterozygous deletion in 11 patients, two of whom also carrying one of the *RHO*-mutations. No mutation was detected in one CSNB-patient suggesting the presence of another mutation segregating in the pedigree.

Conclusions: Here, we present a large family presenting two distinct RD-phenotypes and at least three mutations segregating across three generations. Although further functional studies are needed, this study adds a fifth rhodopsin mutation associated with CSNB. Grant references: KP-06-N33/12/18.12.2019 and D-131/24.06.2020.

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P02.016.A There's more than meets the eye: dual molecular diagnosis in complex hearing loss patients

Beatrice Spedicati[1], *Anna Morgan*[2], *Maria Teresa Bonati*[2], *Giulia Severi*[3], *Agnese Feresin*[1], *Giulia Pelliccione*[2], *Paola Tesolin*[1], *Claudio Graziano*[3], *Paolo Gasparini*[1,2], *Flavio Faletra*[2], *Giorgia Girotto*[1,2]

[1]*Department of Medicine, Surgery and Health Sciences, University of Trieste, Trieste, Italy,* [2]*Medical Genetics, Institute for Maternal and Child Health - I.R.C.C.S. "Burlo Garofolo", Trieste, Italy,* [3]*Medical Genetics Unit, S. Orsola-Malpighi University Hospital, Bologna, Italy.*

Medical geneticists usually try to identify patients' clinical conditions by recognizing the specific pattern of a syndrome. Whenever clinical features do not fit into a known model, the presence of two distinct conditions may be hypothesized and high-throughput sequencing technologies can allow a complete molecular characterization even in the most complex cases. Here we describe patients presenting with hearing loss (HL) and other signs that suggested the presence of a dual molecular diagnosis. Patients were divided in two categories: a) those with distinct phenotypes, i.e. syndromic or non-syndromic HL plus other clinical features related to a second condition; b) patients with overlapping phenotypes, with HL due to either of the two conditions. In group a) **Patient-1** displayed HL and periventricular nodular heterotopia, due to a homozygous deletion of the *STRC* gene and a nonsense variant in the *FLNA* gene, respectively; **Patient-2** was affected by Kabuki syndrome (*KMT2D* gene) and Bosma arhinia microphthalmia syndrome (*SMCHD1* gene); **Patient-3** was affected by Distal renal tubular acidosis with progressive sensorineural HL (*ATP6V1B1* gene) and Marfan syndrome (*FBN1* gene). In group b) **Patient-4** presented sensorineural HL and retinitis pigmentosa: Whole Exome Sequencing revealed two compound heterozygous variants in the *USH2A* gene, responsible for Usher syndrome type 2A, and a nonsense variant in the *EYA4* gene, associated with autosomal dominant non-syndromic HL. Overall, our findings highlighted that "genetic-first diagnostics" should be the gold standard for patients with syndromic conditions of unclear genetic origin, thus avoiding costly and distressing diagnostic procedures before reaching a final diagnosis.

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P02.017.B NGS strategy for the analysis of genes and regions responsible for Early Onset High Myopia

Susana Noval[1], Maria Nieves-Moreno[1], Ana Lopez-Vazquez[1], Fernando Santos-Simarro[2], Irene Rosa-Perez[1], Inmaculada Rueda-Arenas[2], Marta Naranjo-Castresana[2], Agela del Pozo[2], Oriana D'Anna[1], Victoria EF Montaño[2], ***Elena Vallespin***[2]

[1]Department of Ophthalmology, Hospital Universitario La Paz, IdiPaz, Madrid, Spain, [2]Medical and Molecular Genetics Institute (INGEMM), Hospital Universitario La Paz, IdiPaz, Rare Diseases Networking Biomedical Research Centre (CIBERER), ISCIII, Madrid, Spain.

Early Onset High myopia (eoHM) (−6.00 diopters or less) is one of the leading cause of vision loss or even irreversible blindness with pathologic complications such as myopic retinopathy, maculopathy, retinal detachment, cataract or primary open-angle glaucoma that is present before the age of ten. In UMOG (Ophtalmogenetics Multidiciplinary Unit) we have designed and developed a novel and comprehensive screening strategy for all genes and loci responsible for eoHM on next-generation sequencing (NGS) developing a systematic application and automation in the clinical routine. UMOG is a multidisciplinary diagnosis and research team with extensive experience in diagnosis, research and teaching in ophthalmogenetic diseases in Hospital La Paz (Madrid). Samples were screened using a customized NGS gene panel, OFT-v3-1, containing 419 genes associated with eye pathology of suspected genetic origin, including eoHM genes and loci. The patients study was carried out on all the genes in the panel as it has been observed by other researches that a significant proportion of eoHM is caused by mutations in RetNet genes. This panel was validated with at least 25 samples with excellent results. At this point, we already ran 30 eoHM families and the diagnostic yield is around 30%. The development of this project will introduce the use of these new technologies to Health Services for diagnosis and research and thereby will help to improve the diagnosis, treatment and care of patients with this and other genomic disorders. We are grateful to the patients and their families. Grants: PI18-1234-ISCIII and 2020/0197782-ONCE.

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P02.018.C LOXL1 and CACNA1A SNPs associated with exfoliation syndrome susceptibility in a sample of Northern Spanish population

Leire Escudero-Arrarás[1], *Araceli Lara-López[1], María Rodríguez-Hidalgo[1], Txomin Alberdi[2], Iñaki Rodríguez-Agirretxe[2,3,4], Javier Mendicute[2], Javier Ruiz Ederra[5,4]*

[1]Biodonostia Health Research Institute, San Sebastián, Spain, [2]Department of Ophthalmology, Donostia University Hospital, San Sebastián, Spain, [3]Instituto Clínico-Quirúrgico de Oftalmología, Bilbao, Spain, [4]RETICS OFTARED, National Institute of Health Carlos III, Ministry of Economy and Competitiveness, Madrid, Spain, [5]Miramoon Pharma-Biodonostia HRI, San Sebastián, Spain, San Sebastián, Spain.

Introduction: Exfoliation syndrome (XFS) is a systemic disease characterized by whitish fibrillar substance deposition in the anterior segment of the eye. LOXL1 and CACNA1A are the main genes associated with an increased risk.

Materials and Methods: A case-control study with 235 Northern Spanish patients: 55 XFS patients and 180 controls. Genotypes of LOXL1 (rs1048661, rs3825942, rs2165241, rs16958477, rs12914489, rs11638944, rs7173049) and CACNA1A (rs4926244) SNPs were analyzed by direct sequencing.

Results: The G allele and the GG genotype of SNP **rs3825942** were detected at a higher frequency in pseudoexfoliation patients ($p = 0.00057$, $OR = 6.46$; $p = 0.00003$, $OR = 8.96$, respectively). The T allele and the TT genotype of **rs2165241** presented at significantly higher frequencies in XFS patients ($p = 0.00473$, $OR = 2.10$; $p = 0.00001$, $OR = 4.49$, respectively). The G allele and the GG genotype of **rs1048661** were detected at a statistically higher frequency in XFS patients ($p = 0.04587$, $OR = 1.78$; $p = 0.00179$, $OR = 2.66$, respectively). The AA genotype of SNP **rs12914489** was detected at a statistically lower frequency in XFS patients ($p = 0.01898$, $OR = 5.503$). The C allele and the CC genotype of **rs11638944** presented at significantly lower frequencies in pseudoexfoliation patients ($p = 0.00754$, $OR = 0.50$; $p = 0.00876$, $OR = 0.35$, respectively). No significant association between XFS and LOXL1 **rs16958477** and **rs7173049** SNPs and CACNA1A **rs4926244** was observed.

Conclusion: We found a significant association for several LOXL1 SNPs, whereas no differences were attributable to CACNA1A rs4926244 SNP. LEA is supported by fellowships from Fundación Jesús de Gangoiti Barrera and from the Basque Government (2018111062, MTVD19/BD/006 and MTVD20/BD/002). Supported by grants from the ISCIII and FEDER (PI18/00507) and BEGISARE.

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P02.019.D The Fraser-complex pathologic spectrum: Familial cryptophthalmos in two families from GAFSA, TUNISIA

Nouha Bouayed ABDELMOULA, Sonda Kammoun, ***Fatma Abid Mzid****, Saloua Ben Amor, Jamel Feki, Takwa Sammouda, Mouna Rekik*

Genomics of signalopathies at the service of medicine UR17ES36, Medical University of Sfax, Sfax, Tunisia.

Recessive mutations in genes encoding members of the Fraser complex (FC) or associated proteins constitute an established genetic cause of Fraser syndrome in its three forms related to mutations in three different genes FRAS1, FREM2, and GRIP1 resulting in failure of the apoptosis program and disruption of the epithelial-mesenchymal interactions during embryonic development. We report in this study two Tunisian pedigrees from the town of Gafsa in which a recessive cyptophthalmos was detected.

Material and Methods: Two male newborns were referred to our genetic counselling for cryptophthalmos. One of them was also suspected to have Crigler-Najjar disease. Genetic exploration of FRAS1 and UGT1A1 genes was conducted.

Results: The two unrelated males were born to consanguineous parents from Gafsa. They had cryptophthalmos which is a condition of eyelid malformation associated to an underlying malformed eye. The first case had unilateral right complete cryptophthalmos associated with maldevelopment of the cornea and the crystalline as well as microphthalmia. At the left, he had posterior embryotoxon. Besides facial dysmorphism, he had bilateral syndactyly. Molecular analysis showed a homozygosity for an intronic sequence change in intron 22 of FRAS1 gene. The second patient as well as his brother had bilateral complete cryptophthalmos associated to anophthalmia, microphthalmia and iris coloboma. The patient had

also a neonatal jaundice which was related to the homozygous mutation of UGT1A1 exon 3 (c.1070A>G).

Conclusion: More recently, bilateral anophthalmia and liver malformations with intrahepatic biliary atresia were described. FC screening is thus debated for the second family.

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P02.021.B Variants in *FZD5* are primarily associated with non-syndromic phenotypes in individuals with ocular coloboma

Richard J. Holt[1], *David Goudie*[2], *Ajoy Sarkar*[3], *Alejandra Damián*[4,5], *Alejandra Tamayo*[4,5], *Carmen Ayuso*[4,5], *Marta Cortón*[4,5], *Alice Gardham*[6], *Virginia Clowes*[6], *Julie Plaisancié*[7,8], *Nicolas Chassaing*[7,8], *Patrick Calvas*[7,8], *Nicola K. Ragge*[1,9]

[1]*Oxford Brookes University, Oxford, United Kingdom,* [2]*NHS Tayside, Dundee, United Kingdom,* [3]*Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom,* [4]*Instituto de Investigación Sanitaria-Fundación Jiménez Díaz University Hospital - Universidad Autónoma de Madrid, Madrid, Spain,* [5]*Centre for Biomedical Network Research on Rare Diseases, Madrid, Spain,* [6]*Northwick Park and St Mark's Hospital, Harrow, United Kingdom,* [7]*Purpan University Hospital, Toulouse, France,* [8]*Centre Hospitalier Universitaire (CHU) de Toulouse, Toulouse, France,* [9]*Birmingham Women's and Children's Foundation Trust, Birmingham, United Kingdom.*

Introduction: Ocular coloboma results from failure of optic fissure closure during development. It is genetically heterogenous, with variants in 30 genes implicated, many also associated with anophthalmia and microphthalmia. Most recently, heterozygous *FZD5* variants have been reported in individuals with coloboma and microphthalmia, but are limited to frameshifts and inframe insertion/deletions. We describe two missense, two nonsense, and two frameshift variants in individuals with coloboma and/or microphthalmia.

Materials and methods: Variants were identified using whole exome sequencing (WES) or customised NGS panels of ocular development genes, in individuals from the UK (including the DDD Study [www.ddduk.org/access.html]), France and Spain.

Results: We identified six individuals with coloboma and heterozygous likely pathogenic variants: 1) male with bilateral microphthalmia, iris and chorioretinal coloboma, anal atresia, atrial and ventricular septal defect, cortical dysplasia, microcephaly, seizures, deafness, and tracheoesophageal fistula (NM_003468.4:c.539_540insG:p.(E181Rfs*88)), also diagnosed with a pathogenic *SLC12A2* variant (NM_001046:exon4:c.C980T:p.A327V), 2) female with bilateral iris coloboma, patent ductus arteriosus, atrial septal defect, and volvulus (NM_003468:c.C577T:p.(R193C)), 3) female with bilateral iris and optic nerve colobomas, and downslanted palpebral fissures, (NM_003468:c.G1566A:p.(W522*)), 4) female with unilateral iris and chorioretinal coloboma (NM_003468.4:c.541G>T:p.(E181*)), 5) female with bilateral coloboma (NM_003468.4:c.1150G>C:p.(D384H)), and 6) female with unilateral iris and chorioretinal coloboma, and bilateral optic nerve colobomas (NM_003468.4:c.147delG:p.(H50Tfs*70)).

Conclusions: We present six individuals with *FZD5* variants and ocular colobomas, providing the first evidence for *FZD5* missense and stopgain variants in these disorders. As iris coloboma is the only consistent phenotype, these data highlight the importance of additional pathogenic variants underlying associated complex non-ocular phenotypes.

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P02.022.C Clinical exome sequencing reveals different *GUCY2D*-related retinopathies in Bulgarian patients

Kunka Kamenarova[1], *Nevyana Veleva*[2], *Kalina Mihova*[1], *Neda Sergeeva*[2], *Alexander Oscar*[2], *Radka Kaneva*[1]

[1]*Molecular Medicine Center, Medical University – Sofia, Sofia, Bulgaria,* [2]*Department of Ophthalmology, University Hospital "Alexandrovska", Medical University – Sofia, Sofia, Bulgaria.*

Introduction: *GUCY2D* gene encodes the photoreceptor guanylate cyclase (GC-E) and different mutations can lead to cone-rod dystrophy (CRD), congenital stationary night blindness (CSNB), and Leber congenital amaurosis. In this study, we describe three unrelated families who carried different *GUCY2D*-variants and presented two types of retinopathy.

Materials and Methods: Two unrelated patients with autosomal-dominant (ad) and autosomal-recessive (ar) CRD, and one with arCSNB were examined clinically by standard ophthalmological methods. Targeted sequencing of clinical exome on Illumina® platform, followed by Sanger sequencing and segregation analysis, was used to identify pathogenic variants.

Results: All patients manifested decreased vision, photophobia and elevated thresholds of dark adaptation. Genetic analysis revealed three mutations in the *GUCY2D* gene segregating with the phenotype in the pedigrees. The common c.2512C>T (p.Arg838Cys) mutation presenting a relatively severe clinical phenotype of adCRD was found in one of the analyzed families. Mutations c.2900A>G (p.His967Arg) and c.3224+1G>C (p.?) were identified in two different combinations (c.2900A>G/c.3224+1G>C and c.3224+1G>C/c.3224+1G>C) in two unrelated probands affected by arCSNB and arCRD, respectively. *GUCY2D* mutations were accompanied by similar pattern of generalized cone (macular and peripheral) dysfunction with a tendency to less involvement of the rod photoreceptors in the two CRD-patients and a less severe phenotype in the proband with CSNB.

Conclusions: *GUCY2D* is a major gene responsible for progressive CRD which is estimated to affect 1 in 40,000 individuals. Here, we present phenotypes of adCRD, adCRD and arCSNB in three Bulgarian families carrying different pathogenic variants of *GUCY2D*. Grant references: KP-06-N33/12/18.12.2019 and D-131/24.06.2020.

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P02.024.A Benefits of exome sequencing in patients diagnosed with isolated or syndromic hearing loss

Roxane van Heurck, *Maria Teresa Carminho-Rodrigues, Emmanuelle Ranza, Caterina Stafuzza, Lina Quteineh, Corinne Gehrig, Eva Hammar, Michel Guipponi, Marc Abramowicz, Pascal Senn, Nils Guinand, Helene Cao-Van, Ariane Paoloni-Giacobino*

hug, genève, Switzerland.

Purpose: Hearing loss is characterized by an extensive genetic heterogeneity and is a common disorder in children (1/500). Molecular diagnosis is of particular benefit and allows to identify clinically-unrecognized hearing loss syndromes, as well as appropriate management and follow-up, including genetic counselling.

Methods: We performed clinical whole exome sequencing, with analysis of a 189 gene panel associated with hearing loss, in a prospective cohort of 70 patients including 61 children and 9 adults presenting with hearing loss from 2017 to 2020.

Results: The overal diagnostic rate using exome sequencing reached 47,2 % - 50,8% in children and 22% in adults. In children with confirmed molecular results, seventeen out of 31 (54,8%) patients showed autosomal recessive inheritance patterns, thirteen out of 31 (41,94%) showed autosomal dominant and one case had X-linked hearing loss. While in adults the two patients showed autosomal dominant inheritance pattern. Out of the 31 children, 17 (54,84%) had non-syndromic hearing loss and 14 (45,16%) had syndromic hearing loss. Both adult cases were diagnosed with syndromic hearing loss. The most common causative genes were *STRC* (5 cases), *GJB2* (3 cases), *COL11A1* (3 cases), *ACTG1* (2 cases), *GATA3* (2 cases) and *TMPRSS3* (2 cases).

Conclusion: Exome sequencing perfomed in hearing loss situations has a high diagnostic yield in children. This can reveal several hearing loss syndromes before involvement of other organs/systems, thus allowing the surveillance of present and/or future complications associated with these syndromes.

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P02.025.B Auditory development of patients with genetically determined hearing loss

Dominika Ozieblo[1,2], *Anita Obrycka*[3], *Henryk Skarżyński*[4], *Monika Ołdak*[1]

[1]*Department of Genetics, Institute of Physiology and Pathology of Hearing, Warsaw, Poland,* [2]*Postgraduate School of Molecular Medicine, Medical University of Warsaw, Warsaw, Poland,* [3]*Department of Implants and Auditory Perception, Institute of Physiology and Pathology of Hearing, Warsaw, Poland,* [4]*Oto-Rhino-Laryngology Surgery Clinic, Institute of Physiology and Pathology of Hearing, Warsaw, Poland.*

Introduction: Every year, approximately 1-6/1000 children are born with severe to profound hearing loss (HL) and for this group of patients cochlear implantation (CI) is the treatment of choice. The aim of our study was to analyse the auditory development of DFNB1-negative CI patients.

Materials: The study group (n = 52) was recruited from patients with profound prelingual HL that were negative for DFNB1 locus pathogenic variants and had no environmental HL risk factors.

Methods: In all probands exome sequencing (WES) was performed and followed by bioinformatics analysis. Validation of selected variants and family segregation analysis were performed using standard Sanger sequencing or qPCR. Evaluation of patients auditory development was performed with the LittlEARS questionnaire (LEAQ) in three subsequent intervals - at the time of cochlear implant activation as well as in 5th and 9th month after CI.

Results: Causative variants were identified in 69% of patients (36/52). The majority of them are localized in the *MYO15A* (n = 6) and *PAX3* (n = 5) genes. All patients presented a significant improvement of their auditory skills in subsequent intervals at 5th ($p < 0.001$) and $9t^{h}$ ($p < 0.05$) months after CI. There were no statistically significant differences between auditory development of patients with identified and unidentified genetic cause of HL.

Conclusions: Obtained results show a high heterogeneity of genetic HL causes in the population of Polish DFNB1-negative cochlea-implanted patients. All tested children were good candidates for CI as their HL causative genetic variants are localized in genes preferentially expressed in the cochlea. Supported by NCN grant: 2017/27/N/NZ5/02369

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P02.026.C Genetic Variant Curation in *GJB2* and *GJB6* genes from an Argentinean cohort of hearing loss patients

Paula I. Buonfiglio[1], *Carlos D. Bruque*[2], *Sebastián Menazzi*[3], *Liliana Francipane*[3], *Vanesa Lotersztein*[4], *Ana B. Elgoyhen*[1], *Viviana K. Dalamón*[1]

[1]*INGEBI, Ciudad Autónoma de Buenos Aires, Argentina,* [2]*Hospital de Alta Complejidad SAMIC - El Calafate, El Calafate, Argentina,* [3]*Servicio de Genética del Hospital de Clínicas "José de San Martín"., Ciudad Autónoma de Buenos Aires, Argentina,* [4]*Servicio de Genética del Hospital Militar Central Cirujano Mayor "Dr. Cosme Argerich", Ciudad Autónoma de Buenos Aires, Argentina.*

Hereditary hearing impairment affects 1-500 newborn children. It is characterized by the large amount of genes involved (more than 100) and its phenotype heterogeneity. Despite the wide genetic variety of hearing impairment, the most commonly mutated genes in severe to profound autosomal recessive non-syndromic hearing loss are *GJB2* and *GJB6*, accounting for nearly 50% of the cases in most populations around the Mediterranean Sea. Molecular diagnosis enables proper genetic counseling and medical prognosis to patients. Therefore, correct interpretation of the phenotypic consequences of genetic variants is crucial in genetic diagnosis, since discrepancies in sequence variant interpretation and classification has been reported to lead to serious impact in patient health maintenance.In this study we aimed to identify the genetic causes of hearing loss and performed a manual genetic variant curation following the American College of Medical Genetics and Genomics/Association for Molecular Pathology ACMG/AMP standards and hearing-loss-gene-specific criteria of the ClinGen Hearing Loss Expert Panel.A total of 600 patients were studied for genetic variants in *GJB2* and *GJB6* genes by Sanger Sequencing technique and Multiplex Gap-PCR, respectively.Overall, 48 different sequence variants were detected in our cohort of patients, being the c.35delG the most common causative variant identified. Besides, more than 50% of sequence variants were reclassified from their previous categorization in ClinVar after careful manual analysis. These results provide an accurately analysed and interpreted set of variants to be taken into account by clinicians and the scientific community, and hence, aid the precise genetic counseling to patients.

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P02.027.D MYO6 intragenic deletion in a family with autosomal dominant deafness

Chiara Pescucci[1], *Francesca Gerundino*[1], *Costanza Giuliani*[1], *Lucia Galli*[2], *Giuseppina Marseglia*[1], *Barbara Minuti*[1], *Francesca Marin*[1], *Francesca Buchi*[1], *Monica Trafeli*[1], *Paola Pelacani*[1], *Chiara Castagnoli*[1], *Elisabetta Pelo*[1], *Alfredo Orrico*[2,3]

[1]*SOD Diagnostica genetica, AOU Careggi, Firenze, Italy,* [2]*Molecular diagnosis and characterization of pathogenic mechanisms of rare genetic diseases, AOU Senese, Siena, Italy,* [3]*Clinical Genetics ASL Toscana SudEst Ospedale della Misericordia, Grosseto, Italy.*

Introduction: MYO6 loss-of-function variants can cause autosomal dominant progressive hearing loss with variable age of onset. Here we present a family with hereditary hearing loss

transmitted in an autosomal dominant pattern. The proband, a 40-year-old female, began to show a progressive deafness since she was 17 years old. She was diagnosed with sensorineural hearing loss more pronounced in medium and high frequencies (500-8000Hz). Her mother and maternal grandmother also appeared with the same condition with an onset during the second decade.

Materials and Methods: libraries preparation by Illumina TSOne sequencing kit and massive parallel sequencing, followed by bioinformatic analysis of an in-silico panel of hearing loss genes were performed. Reads were aligned to the GRCh37/hg19 using BWA. Single-nucleotide variants and indels were called by SAMtools and GATK. Copy number variants analysis was performed by CONTRA. Variants were functionally annotated by ANNOVAR and interpreted by InterVar. ArrayCGH was perfomed using a custom array specifically designed to investigate intragenic CNVs in hearing loss related genes.

Results: a novel MYO6 intragenic deletion was identified in the proband. Segregation analysis showed that the deletion co-segregates with deafness within the family. ArrayCGH analysis allowed to confirm the presence of a 5,3 kb deletion encompassing exons 2 and 3 of the gene: arr[GRCh37]6q14.1 (76515168x2,76527247_76532572x1,76537323x2).

Discussion: the identification of a novel deletion supports with additional evidence the known matter that a number of pathogenic variants in hereditary deafness are private. As a consequence, molecular diagnosis takes advantage from combined approaches for SNVs/CNVs analyses from sequencing data.

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P02.028.A *GJB2 sequencing, Multiplex Ligation Probe Amplification (MLPA) and Whole Exome Sequencing (WES) for the molecular diagnosis of Non-Syndromic Hearing Loss (NSHL): the experience of a cohort of 277 Italian families*

Anna Morgan[1], *Flavio Faletra*[1], *Stefania Lenarduzzi*[1], *Martina La Bianca*[1], *Giulia Pelliccione*[1], *Beatrice Spedicati*[2], *Agnese Feresin*[2], *Daniela Mazzà*[1], *Alberto Sensi*[3], *Claudio Graziano*[4], *Marco Seri*[4], *Umberto Ambrosetti*[5], *Paolo Gasparini*[1,2], *Giorgia Girotto*[1,2]

[1]*Institute for Maternal and Child Health – IRCCS "Burlo Garofolo", Trieste, Italy,* [2]*University of Trieste, Department of Medicine, Surgery and Health Sciences, Trieste, Italy,* [3]*Medical Genetics Unit, Department of Clinical Pathology, Pievestina, Cesena, Italy,* [4]*Unit of Medical Genetics, S. Orsola-Malpighi Hospital, Bologna, Italy,* [5]*University of Milano U.O.C. Audiologia/Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milano, Italy.*

Introduction: NSHL is the most common sensory disorder, with ~80% of congenital cases due to genetic causes. In addition to screening the most frequently mutated genes (*GJB2*/*GJB6*/*MT-RNR1*), the use of WES together with techniques able to detect copy number variants (CNVs) has proved to be efficient in the molecular diagnosis of NSHL.

Materials and Methods: We applied a multi-step approach for testing 277 NSHL families, which included: 1) an accurate clinical evaluation, 2) the analysis of *GJB2*, *GJB6*, and *MT-RNR1*, 3) the evaluation of *STRC-CATSPER2* and *OTOA* CNVs via MLPA, 4) WES in patients negative to steps 2-3.

Results: About 20% of patients carried mutations in the *GJB2* gene. MLPA and WES led to the characterization of an additional ~37% of cases. In particular, data analysis allowed to 1) confirm the relevant role of CNVs in NSHL, with ~8% of the positive cases carrying a pathogenic CNV, 2) unveil a series of unexpected scenarios, e.g. the detection of syndromes in patients displaying subtle phenotypic features, early diagnosis of late-onset diseases, identify mutations in different genes involved in the same phenotype, detect multiple genetic conditions in the same patient, 3) discover new disease genes (e.g. *PSIP1*, *TBL1Y*, *SPATC1L*, *PLS1*, *SLC12A2*), further exploring the complexity of NSHL.

Conclusions: Our approach proved to be efficient in identifying the molecular causes of NSHL, leading to an overall detection rate of ~50% in the Italian population. Furthermore, WES demonstrated its utility in identifying new disease-genes, deepening the knowledge of the biological mechanisms of NSHL.

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P02.029.B Use of OTO-NGS-v2 panel for the genetic diagnosis of hereditary hearing loss

María Lachgar[1,2], *Matías Morín*[1], *Manuela Villamar*[1], *Miguel Ángel Moreno-Pelayo*[1]

[1]*Hospital Universitario Ramón y Cajal and IRYCIS and CIBERER, Madrid, Spain,* [2]*Wolfson Centre for Age-Related Diseases, King's College London, London, United Kingdom.*

Hereditary hearing loss is the most common sensory deficit in humans. It has a highly heterogeneous genetic aetiology and, therefore, the use of Next Generation Sequencing (NGS) approaches is essential to undertake a genetic diagnosis.In this work, we use OTO-NGS-v2, a custom-designed NGS targeted panel containing 117 genes associated with hearing loss. Library preparation uses IDT probes to capture the regions of interest, followed by sequencing in the Illumina MiSeq and data analysis and variant interpretation in SOPHiA Genetics DDM, with all mutations confirmed by Sanger sequencing.OTO-NGS-v2 was validated using 16 previously genetically characterized samples for the identification of single-nucleotide variants (SNVs), small insertions and deletions (indels), and copy number variations (CNVs). We have studied 108 Spanish families with autosomal dominant sensorineural hearing loss (ADSNHL), 45 of which have been genetically diagnosed, thus constituting a diagnostic rate of 41.67%. The 58.33% of the cases remain undiagnosed and further studies are needed to identify the genetic cause of the hearing impairment in these patients. Our results indicate that *WFS1* and *MYO7A* have the highest prevalence in the Spanish population (6.48%) followed by *MYO6A* (5.56%).We conclude that OTO-NGS-v2 is a robust diagnostic tool for the genetic diagnosis of hereditary hearing loss. In this study, we have laid the foundations for its implementation in clinical practice and contributed to the understanding of the genetic landscape of hearing impairment in the Spanish population.This research was funded by ISCIII (PI17/01659, PI20/0429, CIBERER, 06/07/0036) and by the Regional Government of Madrid (CAM,B2017/BMD3721).

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P02.030.C A *RIPOR2* in frame deletion is a frequent and highly penetrant cause of adult onset hearing loss

Jeroen J. Smits[1], *Suzanne E. de Bruijn*[1], *Chang Liu*[2], *Cornelis P. Lanting*[1], *Andy J. Beynon*[1], *Joëlle Blankevoort*[1], *Jaap Oostrik*[1], *Wouter Koole*[1], *Erik de Vrieze*[1], *DOOFNL Consortium, Cor W. R. J. Cremers*[1], *Frans P. M. Cremers*[1], *Susanne Roosing*[1], *Helger G. Yntema*[1], *Henricus P. M. Kunst*[1], *Bo Zhao*[2], *Ronald J. E. Pennings*[1], *Hannie Kremer*[1]

[1]Radboudumc, Nijmegen, Netherlands, [2]Indiana University School of Medicine, Indianapolis, IN, USA.

Introduction: Hearing loss is one of the most prevalent disabilities worldwide. The adult-onset types of the condition is highly heritable, but the genetic causes are often unknown.

Methods: Family and cohort studies were performed and included exome sequencing and characterization of hearing phenotype. Ex vivo protein expression addressed the functional effect of a DNA-variant.

Results: We identified an in-frame deletion in RIPOR2, that co-segregated with hearing loss in twelve families of Dutch origin in an autosomal dominant pattern. Haplotype analysis indicated the in-frame deletion to be a founder variant, present in 18 of 22,952 individuals of an unselected cohort. This suggests that the deletion is a frequent cause of monogenic hearing impairment in the Netherlands, with potentially 8,000 affected individuals, and a significant cause of hearing impairment in neighboring countries. Hearing loss associated with the deletion in 63 subjects displayed an average age of onset of 30.6 years (SD 14.9 years) and variable audiometric characteristics. A functional effect of the variant was demonstrated by aberrant localization of the mutant RIPOR2 in the stereocilia of cochlear hair cells. Moreover, mutant RIPOR2 failed to rescue the morphological defects observed in RIPOR2-deficient hair cells, in contrast to the wildtype protein.

Conclusion: we identified a relatively common type of inherited hearing loss, with potentially thousands of individuals at risk in the Netherlands and beyond, which makes it an interesting target for developing a (genetic) therapy. This study was financially supported by grants from the DCMN Radboudumc, the Heinsius-Houbolt foundation and NIH/NIDCD (R01 DC017147).

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P02.031.D *GJB2*-associated hearing loss in Northern Ossetians

Nika V. Petrova[1], *Natalia V. Balinova*[1], *Tatyana A. Vasilyeva*[1], *E F. Mikhailidi*[2], *Vitaliy V. Kadyshev*[1], *Rena A. Zinchenko*[1,3]

[1]Research Centre for Medical Genetics, Moscow, Russian Federation, [2]Republican Children's Clinical Hospital, North Ossetia-Alania, Vladikavkaz, Russian Federation, [3]N.A. Semashko National Research Institute of Public Health, Moscow, Russian Federation.

Hearing loss (HL) is the most common sensorineural disorder worldwide. Pathogenic variants in the *GJB2* gene are the main cause of congenital deafness in different populations. The aim was to determine the contribution of the GJB2 gene to the hereditary sensorineural hearing loss (HSNHL) incidence in Ossetians, including Ironians and Digorians, the main subethnic groups, from North Ossetia-Alania. Molecular genetic testing (sequencing and MLPA) was performed in 65 HL patients. In 27.7% HSNHL was associated with *GJB2* variants. The c. 358_360delGAG variant frequency was 42.5% (19/42) in Ossetians with *GJB2*-associated HL, and 15.4% (20/130) in the general sample. The c.35delG variant accounts for 38.1% (16/42) in Ossetian patients with *GJB2*-associated HL and 12.3% (16/130) in the total sample. 368 healthy Northern Ossetian individuals, including 248 Ironians and 65 Digorians, were analyzed for variants c. 358_360delGAG and c.35delG. The frequencies of c.35delG and c.358_360delGAG *GJB2* variants were 0.0061 and 0.0121 in Ironians, 0.0077 and 0.0154 in Digorians. Less than 30% of cases of hereditary sensorineural hearing loss in Ossetians were *GJB2*-associated. Also, as in other North Caucasian populations (for example, in Karachai), in Ossetians the most frequent *GJB2* variant was c. 358_360delGAG. Two variants, c.358_360delGAG and c.35delG, made up 90% of alleles in *GJB2*-associated hearing loss in Ossetians. The summarized frequency of *GJB2* pathogenic variants in Ossetian population exceeds 2% (1.8% in Iranians and 2.3% in Digorians). The research was partially supported by RSF grant №17-15-01051 and within the state task of the Ministry of education and science of Russia.

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P02.032.A A novel compound heterozygous mutation in *RBP3* causes High Myopia

Maya Gombosh[1], *Yuval Yogev*[1], *Noam Hadar*[1], *Libe Gradstein*[2], *Ohad S. Birk*[3,1]

[1]The Morris Kahn Laboratory of Human Genetics, National Institute of Biotechnology in the Negev, Beer Sheva, Israel, [2]Department of Ophthalmology, Soroka Medical Center, Beer Sheva, Israel, [3]Genetics Institute, Soroka Medical Center, Beer Sheva, Israel.

We studied a girl presenting with isolated high myopia. Whole exome sequencing was done and data were analyzed using our in-house tool, filtering through our in-house 582 ethnicity-matched controls and available databases, based on allele frequency, linkage locus, indel mutation analysis, etc. SNP arrays (750K) of family members yielded possible disease-associated loci on chromosomes 6 and 10. A single heterozygous missense mutation (c.3341G>A ; p. p.Arg1114Gln) in *RBP3* was found within the chromosome 10 locus, analyzed using our in-house databases along with open access databases and verified through Sanger sequencing. In addition, CMA identified a ~5 million bp heterozygous deletion, encompassing *RBP3*, within that locus. Integrative Genomics Viewer (IGV) analysis of NGS data was used to confirm the deletion mutation, showing ~50% less reads in the deleted region compared to controls. Segregation analysis demonstrated that the missense mutation was inherited from the heterozygous father and that the deletion mutation was de-novo. Thus, the infantile high myopia phenotype was caused by novel compound heterozygous *RBP3* mutations: an inherited heterozygous missense mutation and a large de-novo deletion mutation encompassing *RBP3*, that was identified through Indel analysis and low levels of NGS reads of the patient compared to controls. This is a first report of a large deletion mutation in *RBP3*, which we show to be an unusual "second hit" de-novo germline mutation. Genetic diagnosis is important in children presenting with infantile high myopia, which can be the presenting sign of a degenerative ocular disorder.

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P02.033.B Differential methylation of microRNAs encoding genes may contribute to high myopia

Joanna Swierkowska[1], *Sangeetha Vishweswaraiah*[2], *Justyna A. Karolak*[3,1], *Malgorzata Mrugacz*[4], *Uppala Radhakrishna*[2], *Marzena Gajecka*[1,3]

[1]Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland, [2]Department of Obstetrics and Gynecology, Oakland University William Beaumont School of Medicine, Royal Oak, MI, USA, [3]Chair and Department of Genetics and Pharmaceutical

Microbiology, Poznan University of Medical Sciences, Poznan, Poland, [4]Department of Ophthalmology and Eye Rehabilitation, Medical University of Bialystok, Bialystok, Poland.

Introduction: High myopia (HM), an eye disorder with a refractive error of -6.0 D or higher, has multifactorial etiology with environmental and genetic factors involved. Research evidence supports the contribution of alterations in DNA methylation and genes encoding microRNAs (miRNAs) to myopia pathogenesis. Here, we combined both aspects to study the role of the miRNA gene methylation in HM.

Materials and Methods: From genome-wide methylation results of blood DNA of 18 Polish children with HM and 18 matched controls, we retrieved differentially methylated CG dinucleotides located in miRNA genes. Those miRNA genes and their targets were included in over-representation analyses in ConsensusPathDB-human. Expression of selected miRNAs' target genes were also assessed using the RNA-seq data of human retinal ARPE-19 cell line.

Results: Significant differential methylation of CG dinucleotides located in the promoter regions of *MIR3621*, *MIR34C*, *MIR423* (increased methylation level), and *MIR1178*, *MIRLET7A2*, *MIR548I3*, *MIR6854*, *MIR675*, *MIRLET7C*, *MIR99A* (decreased methylation level) genes could alter their expression. Several targets of those miRNAs, e.g. *NAP1L1* and *EIF4B*, were highly expressed in the retinal cell line. Over-representation analyses of miRNAs' genes and their targets revealed enrichment in biological pathways related to eye structure and function, such as Wnt signaling, axon guidance, and insulin signaling.

Conclusions: Differential methylation of promoters of indicated miRNAs' genes might influence their expression. Therefore, it may contribute to HM pathogenesis via the disrupted regulation of transcription of miRNAs' target genes and biological pathways crucial for eye development and function.

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P02.035.C Genetics of Pain: Novel variants identified by the European Network on Inherited Sensory Neuropathies and Insensitivity to Pain (ENISNIP)

Annette Lischka[1], *Katja Eggermann*[1], *Arman Çakar*[2], *Richard Boček*[3], *Luca Bartesaghi*[4], *Miriam Elbracht*[1], *Thorsten Hornemann*[5], *Jan Senderek*[6], *Michaela Auer-Grumbach*[7], *Yesim Parman*[2], *Petra Laššuthová*[3], *Ingo Kurth*[1]

[1]*Institute of Human Genetics, RWTH Aachen University Hospital, Aachen, Germany,* [2]*Neurology Department, Neuromuscular Unit, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey,* [3]*Department of Pediatric Neurology, 2nd Faculty of Medicine, Charles University, Prague, Czech Republic,* [4]*Department of Neuroscience and Department of Clinical Neuroscience, Karolinska Institute, Stockholm, Sweden,* [5]*Institute for Clinical Chemistry, University Hospital Zurich, Zurich, Switzerland,* [6]*Friedrich-Baur-Institute at the Department of Neurology, University Hospital LMU Munich, Munich, Germany,* [7]*Division of Orthopedics, Medical University of Vienna, Vienna, Austria.*

Introduction: Mutations in approximately 20 genes lead to a monogenetic disorder of lack of pain perception. This includes clinical entities such as hereditary sensory and autonomic neuropathies (HSAN) and congenital insensitivity to pain (CIP). Clinically, the various disorders manifest themselves through repeated trauma and mutilation. Yet, small individual patient cohorts and the lack of standardized phenotype information hinder the complete elucidation of these genetic disorders.

Materials and Methods: The European Network on Inherited Sensory Neuropathies and Insensitivity to Pain (ENISNIP) was established by seven research centers and two patient advocacy organizations specialized on HSAN/CIP and accumulates the knowledge from clinicians, geneticists, basic scientists and patients. The exomes of 60 HSAN patients and, if available, unaffected family members were sequenced. Genetic and clinical data were shared and harmonized within the network.

Results: We identified 16 likely disease-causing novel variants in the following HSAN/CIP genes: *ATL3, DST, FLVCR1, NGF, NTRK1, PRDM12, SCN9A, SPTLC2* and *WNK1*. All variants were rare or absent from control cohorts and none had previously been reported in the literature. If applicable, the pathogenicity was corroborated by segregation analyses within the families.

Conclusions: Through compiling the data within the ENISNIP network, here we report on 16 patients with novel pathogenic variants in known HSAN/CIP genes. In a next step, the data of genetically unsolved cases will be harmonized and re-evaluated. We will prospectively recruit and analyze additional patients to identify new disease-relevant genes.

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P02.036.A Genetics of Inherited Retinal Degenerations in Icelandic patients

Daniel A. Thorsteinsson[1], *Vigdis Stefansdottir*[2], *Sigridur Thorisdottir*[2], *Thor Eysteinsson*[1], *Jon J. Jonsson*[1,2]

[1]*Univ. of Iceland, Reykjavik, Iceland,* [2]*Landspitali - National University Hospital of Iceland, Reykjavik, Iceland.*

Introduction: The study objective was to delineate the genetics of inherited retinal degenerations (IRDs) in Iceland, a small nation of 364.000 and a genetic isolate. Benefits include delineating novel pathogenic genetic variants and defining genetically homogenous patients as potential investigative molecular therapy candidates.

Materials and Methods: The study sample comprised patients with IRD in Iceland ascertained through national centralized genetic and ophthalmological services at Landspitali, a national social support institute, and the Icelandic patient association. Information on patients' disease, syndrome, and genetic testing was collected in a clinical registry. Variants were reevaluated according to ACMG/AMP guidelines.

Results: Overall, 140 IRD patients were identified (point prevalence of 1/2.600), of which 70 patients had a genetic evaluation where two-thirds had an identified genetic cause. Thirteen disease genes were found in patients with retinitis pigmentosa, with the *RLBP1* gene most common (n = 4). The c.1073+5G>A variant in the *PRPF31* gene was homozygous in two RP patients. All tested patients with X-linked retinoschisis (XLRS) had the same possibly unique *RS1* pathogenic variant, c.441G>A (p.Trp147X).

Conclusion: Pathologic variants and genes for IRDs in Iceland did not resemble those described in ancestral North-Western European nations. Four variants were reclassified as likely pathogenic. One novel pathogenic variant defined a genetically homogenous XLRS patient group. **Grants**. Icelandic Student Innovation Fund no. 2107575389. The Richard P. Theodore and Dora Sigurjonsdottir Fund for improving scientific knowledge on blindness no. 2509962099.

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P02.037.B The genetic testing landscape for inherited retinal diseases in the European region

Orla Galvin, *Petia Stratieva, Avril Daly*

Retina International, Dublin, Ireland.

While potential treatments emerge for Inherited Retinal Diseases (IRDs), the genetic characteristics of IRDs require a genetic diagnosis for inclusion in clinical trials. With a genetic diagnosis patients can take action on inheritance patterns and disease progression. To advocate for equitable, affordable, accessible and timely genetic testing for IRDs it was necessary to investigate the genetic testing landscape from a processes and systems perspective.Desktop research supplemented by survey of ophthalmic and/or genetic specialists across 18 countries was employed. Information was provided by: Medical Geneticists, Clinical Laboratory Geneticists, Ophthalmologists, Retinal Specialists. Genetic testing and counselling for IRDs vary substantially among countries from an awareness, accessibility and affordability perspective. Methods of genetic testing vary and include cerebral MRI, Sanger sequencing or Next Generation Sequencing, Whole Exome Sequencing or Whole Genome Sequencing, SNP array or MLPA. Affordability is a barrier for patients in countries without payment schemes (e.g., Poland) and where only targeted population is covered (e.g., Bulgaria). Research project parfticipation is in some regions an alternative, however limited, with patients often advised to send samples for examination abroad, or travel themselves for examination outside their country of origin. Huge disparity exists in the approach to genetic testing for IRDs. Greater awareness of genetic testing services is required among the health sector and eyecare professionals. A revised approach to the provision of genetic testing services is required to ensure equitable access, empower patients, improve access to clinical trials, therapy and delivery of care.Funded by an educational grant from the Allergan Foundation.

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P02.038.C Mutation spectrum of inherited retinal dystrophies in a cohort from the Basque Country (Spain)

María Rodríguez Hidalgo[1], *Maitane Ezquerra-Inchausti*[1], *Leire Escudero-Arrarás*[1], *Araceli Lara-López*[1], *Marta Galdós*[2], *Maialen Aldazabal*[3], *Gonzaga Garay-Aramburu*[3], *Carlos Cruchaga*[4], *Cristina Irigoyen*[5], *Javier Ruiz-Ederra*[6,7]

[1]*IIS Biodonostia, San Sebastián, Spain,* [2]*Cruces University Hospital, Bilbao, Spain,* [3]*Araba University Hospital, Vitoria-Gasteiz, Spain,* [4]*Washington University School of Medicine, St. Louis, MO, USA,* [5]*Donostia University Hospital, San Sebastián, Spain,* [6]*Miramoon Pharma-Biodonostia HRI, San Sebastián, Spain,* [7]*RETICS OFTARED, Madrid, Spain.*

Introduction: Inherited retinal dystrophies (IRD) are a heterogeneous group of diseases that mainly affect the retina, with more than 250 genes involved. The clinical and genetic heterogeneity complicates the identification of causative mutations. Here we present the results of genetic-molecular characterization in a cohort of Basque patients.

Materials and Methods: A retrospective study was carried out on 744 IRD affected individuals (from 266 unrelated families) using different molecular techniques, including gene panel, whole exome sequencing, and MLPA hybridation arrays.

Results: Overall, 50% (133/266) of the studied families were genetically characterized. 126 likely causative variants were identified. Most variants, 81, were missense/nosense; 28 small insertion/deletions, 12 affected splice regions, 4 involved copy number variations and 1 was a complex rearrangement. These variants were identified in 42 genes. The most recurrently mutated genes were *USH2A, CERKL and RHO*, in 21, 10 and 10 families respectively. Most frequent pathogenic variants were c.2276G>T (p.Cys759Phe) in *USH2A, c.847C>T* (p.Arg283Ter) in *CERKL* and c.3260C>T (p.Ser1087Leu) in *SNRNP200*, identified in 12, 10 and 8 families respectively.

Conclusions: Our study allowed us to characterize 50% of the families in our cohort, having important implications for genetic diagnosis and counselling to the Basque population. MRH is supported by a fellowship from Gobierno Vasco, Spain (Pre-2019-1-0325). Work supported by grants from the Instituto de Salud Carlos III y fondos FEDER (PI17/01413 and PI20/01186), from Gobierno Vasco (2018111062, MTVD19/BD/006, MTVD20/BD/002, ZL-2020/00780) and from the Foundation of Patients of Retinitis Pigmentosa (BEGISARE).

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P02.039.D *RPE65*-related retinal dystrophy: mutational and phenotypic spectrum in 45 affected patients

Rosario Lopez-Rodriguez[1], *Esther Lantero*[1], *Fiona Blanco-Kelly*[1], *Almudena Avila-Fernandez*[1], *Inmaculada Martin Merida*[1], *Marta del Pozo-Valero*[1], *Irene Perea-Romero*[1], *Olga Zurita*[1], *Belén Jiménez-Rolando*[2], *Saoud Tahsin Swafiri*[1], *Rosa Riveiro-Alvarez*[1], *María José Trujillo-Tiebas*[1], *Ester Carreño Salas*[2], *Blanca García-Sandoval*[2], *Marta Corton*[1], *Carmen Ayuso*[1]

[1]*Department of Genetics & Genomics, Instituto de Investigación Sanitaria-Fundación Jiménez Díaz University Hospital- Universidad Autónoma de Madrid (IIS-FJD, UAM), Centre for Biomedical Network Research on Rare Diseases (CIBERER), Madrid, Spain,* [2]*Department of Ophthalmology, Fundación Jiménez Díaz University Hospital (FJD), Madrid, Spain.*

Introduction: Biallelic pathogenic *RPE65* variants are related to a spectrum of clinically overlapping inherited retinal dystrophies (IRD). A better knowledge of the mutational spectrum and the phenotype-genotype correlation in *RPE65*-related IRD is needed.

Materials and Methods: Forty-five affected subjects from 27 unrelated families with *RPE65*-related IRD were included. Clinical evaluation consisted on self-reported ophthalmological history and objective ophthalmological examination. Patients' genotype was classified accordingly to variant class (truncating or missense) or to variant location at different protein domains. Main phenotypic outcome was age at onset (AAO) of the symptomatic disease and a Kaplan-Meier analysis of disease symptom event-free survival was performed.

Results: Twenty-nine different *RPE65* variants were identified in our cohort, 7 of them novel. Most frequent variants were p.(Ile98Hisfs*26), p.(Pro111Ser) and p.(Gly187Glu) accounting for the

24% of the detected alleles. Patients carrying two missense alleles showed a later disease onset than those with 1 or 2 truncating variants. While the 60% of patients carrying a missense/missense genotype presented symptoms before or at the first year of life, almost all patients with at least 1 truncating allele (91%) had an AAO ≤1 year (Log Rank test $p < 0.05$).

Conclusion: Our findings suggest an association between the type of the *RPE65* carried variant and the AAO, providing useful data for clinical management of these patients.

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P02.040.A Expanding genetic investigation in patients with isolated foveal hypoplasia

Lucia Tiberi[1], *Camilla Rocca*[1], *Angelica Pagliazzi*[2], *Giovanna Traficante*[2], *Giacomo Bacci*[3], *Elisa Marziali*[3], *Pina Fortunato*[3], *Debora Vergani*[2,1], *Daniela Formicola*[2], *Viviana Palazzo*[2], *Rosangela Artuso*[2], *Samuela Landini*[2,1], *Laura Giunti*[2,1], *Elia Dirupo*[2], *Sara Bargiacchi*[2]

[1]*University of Florence, Department of Experimental and Clinical Biomedical Sciences, Florence, Italy,* [2]*Meyer Children's hospital, Medical Genetics Unit, Florence, Italy,* [3]*Meyer Children's Hospital, Pediatric Ophthalmology Unit, Florence, Italy.*

Foveal Hypoplasia is a rare ocular malformation that can be associated with variable ocular features such as congenital nystagmus, premature cataract, anterior segment dysgenesis and chiasmal misrouting, or either can be found in the context of aniridia, albinism or retinal dystrophy. To date, only *PAX6*, *SLC38A8* and *AHR* are known to be associated with Isolated Foveal Hypoplasia (FVH), but few patients undergo extensive clinical and molecular investigations. In order to elucidate the genetic background of FVH, we performed WES in eight trios recruited at Meyer Children's Hospital in Florence. The patients were phenotypized by ophthalmologist and geneticist and they all presented FVH with no clear iris transillumination defects. In five patients, WES revealed a combination of two *in cis* polymorphisms that are in trans with another *TYR* deleterious mutation: this triallelic genotype, already known to explain missing heritability in some OCA1B albinism patients, is likely to explain also part of FVH patients. One patient showed compound heterozygosity in *TYR*, with one frameshift and one rare missense variant. Another case presented compound heterozygous variants in *OCA2*. Only in one patient we couldn't reach molecular diagnosis, although variants in *TYR* and *OCA2* were found: nonetheless, no clear support of a possible digenic condition was found in the literature. Our results revealed that patients with FVH harbour mutations in genes classically associated to albinism, suggesting that FVH and OCA could be considered as part of the same spectrum. However, further studies in a larger cohort are necessary to better characterize genotype-phenotype correlation.

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P02.041.A Cone-related transcriptomic profiles in Keratoconus corneal epithelium

Katarzyna Jaskiewicz[1], *Magdalena Maleszka-Kurpiel*[2,3], *Malgorzata Rydzanicz*[4], *Justyna A. Karolak*[5], *Rafał Płoski*[4], *Marzena Gajecka*[1,5]

[1]*Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland,* [2]*Optegra Eye Health Care Clinic, Poznan, Poland,* [3]*Chair of Ophthalmology and Optometry, Poznan University of Medical Sciences, Poznan, Poland,* [4]*Department of Medical Genetics, Medical University of Warsaw, Warsaw, Poland,* [5]*Chair and Department of Genetics and Pharmaceutical Microbiology, Poznan, Poland.*

Introduction: Keratoconus (KTCN) is the most common corneal ectasia, affecting 1:2000 individuals worldwide, characterized by progressive thinning of the cornea leading to pathological cone formation. Since corneal epithelium (CE) thinning and CE thickness asymmetry constituting characteristic pattern were observed in KTCN, we aimed to analyze transcriptomic profiles of three distinct CE regions.

Materials and Methods: The 17 KTCN patients undergoing cross-linking procedure and 5 mild myopia patients undergoing refractive error correction (non-KTCN controls) were ascertained. The central, middle and periphery CE regions were separated from each obtained CE, based on CE thickness mapping. The RNA samples were extracted using RNA/DNA/Protein PurificationPlus MicroKit (NorgenBiotek). The NGS libraries were prepared using TruSeq Stranded TotalRNA LibraryPrep Gold (Illumina) and sequenced on Illumina NovaSeq6000 platform (100mln read pairs per sample). RNA-seq data was analyzed implementing previously established pipelines.

Results: We observed a characteristic doughnut pattern (thin cone center surrounded by thickened annulus) on each ectatic epithelial thickness map. The average CE thickness values of the three analyzed regions were found to be statistically different in KTCN patients. Those irregularities in epithelial thickness profiles in KTCN were reflected in transcriptomic profiles. The identified differentially expressed pathways (extracellular matrix organization, epithelial-mesenchymal transition) were consistent with previously reported as dysregulated in KTCN.

Conclusions: The phenotypic abnormalities in distinct KTCN CE regions are related to the identified transcriptomic alterations.

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P02.042.C Assessing the potential of next-generation sequencing technologies to unravel the molecular spectrum of maculopathies

Marta del Pozo-Valero[1,2], *Rosa Riveiro-Álvarez*[1,2], *Inmaculada Martín-Mérida*[1,2], *Fiona Blanco-Kelly*[1,2], *Saoud Swafiri*[1,2], *Isabel Lorda-Sanchez*[1,2], *M Jose Trujillo-Tiebas*[1,2], *Ester Carreño*[3], *Belén Jiménez-Rolando*[3], *Blanca García-Sandoval*[4,2], *Marta Corton*[1,2], *Almudena Avila-Fernandez*[1,2], *Carmen Ayuso*[1,2]

[1]Department of Genetics, IIS-FJD, UAM, Madrid, Spain, [2]CIBERER, Madrid, Spain, [3]Department of Ophthalmology, IIS-FJD, UAM, Madrid, Spain, [4]Department of Opthalmology, IIS-FJD, UAM, Madrid, Spain.

Introduction: Inherited macular dystrophies (MD) comprise a heterogeneous group of disorders characterized by bilateral central visual loss and atrophy of the macula and underlying retinal pigment epithelium. The aim of this study is to assess the potential of next-generation sequencing (NGS) technologies to characterize MD patients.

Methods: The cohort was classified according to their suspected clinical diagnosis- Stargardt disease (STGD), cone and cone-rod dystrophy (CCRD) or other maculopathies (otherMD). Unsolved cases without NGS were re-studied mainly by exome sequencing.

Results: With the implementation of exome sequencing and the study of intronic regions of *ABCA4*, a total of 677 patients (65%) were characterized. The re-study of unsolved cases showed a characterization yield of 63%, including 75% of monoallelic STGD cases in which a second pathogenic variant was found. While most of the patients referred with STGD were characterized with *ABCA4*, most of patients with otherMD were unsolved. Other MD- related (*BEST1*, *PROM1*, *PRPH2)* but also unrelated (*RHO, RPGR*) genes were involved.

Conclusions: This study provides a genetic landscape of 1036 arMD families, giving a mutational spectrum of the genes involved in STGD, CCRD and otherMD groups of patients according to their suspected diagnosis. We demonstrate the increase of the characterization diagnostic yield after the implementation of exome sequencing regardless their diagnosis together with the analysis of the intronic region of *ABCA4* in monoallelic STGD patients. This allows their genetic characterization, even when no clinical and familiar data are available, leading to their reclassification. **Funding**: ISCIII, CIBERER, ONCE, UniversityChairUAM-IIS-FJD, RAREGenomics-CM

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P02.044.A A burden of rare missense variants supports *OTOG* as a frequent gene in familial Meniere disease

Pablo Román-Naranjo[1], *Paula Robles-Bolivar*[1], *María del Carmen Moleón*[2], *Andrés Soto-Varela*[3], *Ismael Arán*[4], *Juan Manuel Espinosa-Sánchez*[2], *Juan Carlos Amor-Dorado*[5], *Angel Batuecas-Caletrio*[6], *Paz Perez-Vazquez*[7], *Alvaro Gallego-Martinez*[1], *Jose Antonio Lopez-Escamez*[1,2,8]

[1]Centre for Genomics and Oncological Research (GENYO), Granada, Spain, [2]Department of Otolaryngology, Instituto de Investigación Biosanitaria, ibs.GRANADA, Hospital Universitario Virgen de las Nieves, Granada, Spain, [3]Division of Otoneurology, Department of Otorhinolaryngology, Complexo Hospitalario Universitario, Santiago de Compostela, Spain, [4]Department of Otolaryngology, Complexo Hospitalario de Pontevedra, Pontevedra, Spain, [5]Department of Otolaryngology, Hospital Can Misses, Ibiza, Spain, [6]Department of Otolaryngology, Hospital Universitario Salamanca, Instituto de Investigación Biomédica de Salamanca (IBSAL), Salamanca, Spain, [7]Department of Otorhinolaryngology, Hospital Universitario de Cabueñes, Gijón, Spain, [8]Department of Surgery, Division of Otolaryngology, Universidad de Granada, Granada, Spain.

Introduction: Meniere disease is a set of a rare inner ear disorder characterized by sensorineural hearing loss, vertigo, and tinnitus. Several genes, including *FAM136A*, *DTNA*, *PRKCB* or *SEMA3D* have been found in autosomal dominant familial Meniere's disease (FMD). The aim of this study was to investigate 73 FMD patients (46 families) to search for new genes in FMD.

Materials and Methods: Rare variants were selected from patient exome sequencing data to perform a gene burden analysis. Allelic frequencies were compared with European and Spanish reference datasets. Only genes with at least 3 rare variants (MAF≤0.05) were retrieved.

Results: We observed an enrichment of rare missense variants in *OTOG* gene in FMD cases against either Non-Finnish European population from gnomAD ($OR = 4.0(2.6\text{-}6.1)$, $p = 4.6x10^{-9}$) or Spanish population ($OR = 3.0(1.9\text{-}4.8)$, $p = 1.2x10^{-5}$). Ten rare missense variants were identified in 15/46 (33%) families with MD in the *OTOG* gene. Six families with 2 or more shared variants were identified, suggesting compound heterozygous recessive inheritance (Table).

Conclusions: OTOG is a frequent gene in FMD and some families with shared variants showed an autosomal recessive inheritance consisting of compound heterozygous missense variants.

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P02.045.B Novel stopgain variant in *SOX2* gene causing autosomal dominant type 3 syndromic microphthalmia

Florina Stoica[1], *Adela Chirita-Emandi*[2], *Andreea Ionescu*[2], *Nicoleta Andreescu*[2], *Maria Puiu*[2]

[1]Ophthalmology Department, Emergency Clinical Municipal Hospital, Center of Genomic Medicine, University of Medicine and Pharmacy "Victor Babes", Timisoara, Romania, [2]Center of Genomic Medicine, University of Medicine and Pharmacy "Victor Babes", Regional Center of Medical Genetics Timis, Clinical Emergency Hospital for Children "Louis Turcanu", part of ERN ITHACA, Timisoara, Romania.

We aim to present a novel variant in *SOX2* gene associated with a particular ocular phenotype in a child with AD type 3 syndromic microphthalmia.

Methods: The patient was evaluated by a multidisciplinary team. NGS used the TruSightOne Illumina gene panel (4813 genes), supported by our Ministry of Health.

Results: Ocular phenotypes in the 4 years old male patient included bilateral microphthalmia, retinal detachment, iris and chorioretinal coloboma, retinal dystrophy and severe visual impairment. The left eye presented high myopia and retinal pigment deposits. Head MRI showed optic nerve and chiasma hypoplasia. Other abnormalities were: mild intellectual disability, epilepsy, walking difficulty, leg bone pain, genu varrum, pes planus, and patent foramen ovale. He had normal male genitalia and height at 50th percentile for age. The endocrinologist monitors the child's growth. NGS performed at 4 years of age detected a heterozygous stopgain variant NM_003106.3*(SOX2)*: c.600C>A, (chromosome 3q26.33). This sequence change creates a premature translational stop signal NP_003097.1:p.(Tyr200Ter), classified as pathogenic.

Conclusions: Heterozygous pathogenic variants in SOX2 gene are associated with syndromic microphthalmia, including ocular and systemic abnormalities. Syndromic microphthalmia diagnosis

Rare variants found in OTOG gene in familial MD cases.

Variant position	Families	MAF FMD	MAF NFE		MAF CSVS	CADD	Domain	AA change
			ExAC	GnomAD				
11:17574758G>A	F1; F14	0.021 (3/73)	0.00080	0.0011	0.0033	24.8	vWD	V141M
11:17578774G>A	F2; F3; F4; F5	0.034 (5/73)	0.0090	0.0041	0.017	15.95	vWD	V269I
11:17594747C>A	F34	0.013 (2/73)	—	—	—	22.2	C8	P747T
11:17621218C>T	F6; F7	0.013 (2/73)	0.0026	0.0058	0.0033	34	C8	P1240L
11:17627548G>A	F14	0.012 (1/73)	0.0056	0.0045	0.0054	23.6	Abf	R1353Q
11:17631453C>T	F8	0.0069 (1/73)	0.017	0.011	0.014	12.89	—	L1548F
11:17632921C>T	F2; F3; F4; F5	0.034 (5/73)	0.0015	0.0011	0.0054	7.71	—	A2037V
11:17656672G>A	F10	0.0069 (1/73)	0.0034	0.0052	0.0039	31	—	R2556Q
11:17663747G>A	F1; F13; F14	0.027 (4/73)	0.0058	0.0024	0.0054	19.41	—	R2802H
11:17667139G>C	F9; F11; F12	0.041 (6/73)	0.019	0.023	0.017	27.2	CT	K2842N

was considered since patient's birth, yet NGS was performed at 4 years, showing that access to molecular diagnosis is still limited in Romania. The novel pathogenic variant identified in the patient was associated with a particular phenotype specifically in regards to retinal pigment deposits, yet with normal male genitalia. The molecular diagnostic was important for the patient to receive specialized care, while the family to benefit from genetic counselling.

F. Stoica: None. **A. Chirita-Emandi:** None. **A. Ionescu:** None. **N. Andreescu:** None. **M. Puiu:** None.

P02.046.C The role of BMP3 in the development of myopia

Amy Findlay, *Thibaud Boutin, Chloe Stanton, Ian Jackson, Veronique Vitart*

MRC Human Genetics Unit, University of Edinburgh, Edinburgh, United Kingdom.

Our research focuses on functional follow up of GWAS hits using a variety of cell and animal models. A locus overlapping the BMP3 gene, significantly associated with retinal detachment risk and myopia, was further examined. Within the associated region, a BMP3 coding variant was prioritized as the candidate causal variant, alongside non-coding potentially regulatory variants. The BMP signalling pathway has long been implicated in the patterning and development of the eye, but BMP3's role both within this pathway and during eye development and disease is unclear. The region of interest is conserved between mouse and human so producing animal models carrying the missense variant along with loss of function mutations was easily accomplished using CRISPR cas9 genome editing and comprehensive phenotyping was performed. Our unit is uniquely equipped to analyse eye phenotypes and using the Optical Coherence Tomography machine we are able to track disease progression throughout life and show that loss of function mice exhibit myopia. RNAseq analysis of loss of function cell lines has shown deregulation of a number of genes including BMP2, and 4, which have also been implicated in myopia. Future work is required to assess how much the mouse and human cell line results converge, but our results strengthen a role for a BMP pathway in myopia development.

A. Findlay: None. **T. Boutin:** None. **C. Stanton:** None. **I. Jackson:** None. **V. Vitart:** None.

P02.047.D Next-generation sequencing panels for hereditary hearing loss testing with approaches for difficult-to-sequence regions

Laura Sarantaus[1], *Kimberly Gall*[2], *Sari Tuupanen*[1], *Marta Gandia*[1], *Hatice Duzkale*[1], *Inka Saarinen*[1], *Johanna Sistonen*[1], *Juha Koskenvuo*[1], *Tero-Pekka Alastalo*[2]

[1]*Blueprint Genetics, a Quest Diagnostics Company, Espoo, Finland,* [2]*Blueprint Genetics Inc, a Quest Diagnostics Company, Seattle, WA, USA.*

Introduction: Hereditary hearing loss (HHL) is a genetically heterogeneous group of disorders. Determining the molecular etiology allows for personalised patient management and surveillance, and recurrence risk estimation for families. Comprehensive genetic testing in HHL using next-generation sequencing (NGS) is complicated owing to pseudogenes and segmental duplications affecting several key genes, such as *STRC*. A testing strategy that includes difficult-to-sequence regions is needed to maximise diagnostic yield.

Materials and Methods: To assess the diagnostic efficacy, we performed a retrospective review of test results from NGS panels performed for 934 de-identified patients tested consecutively for HHL at Blueprint Genetics, a CLIA-certified diagnostic laboratory. The analysis was boosted with clinically relevant deep intronic variants and a proprietary copy number variation (CNV) analysis method for exon-level CNVs. Variant interpretation followed ACMG guidelines. Most cases (86%, 799/934) were analysed using the Comprehensive Hearing Loss and Deafness Panel (239 genes), while a minority (14%, 135/934) were analysed using smaller panels.

Results: Molecular genetic diagnosis was established in 29.9% (279/934) of patients, distributed in 54 genes. The most commonly implicated genes were *GJB2* (24.4%), *STRC* (9.7%), *MYO15A* (5.0%), and *SLC26A4* (4.7%). CNVs were reported in 16.1% (45/279) of diagnosed patients; 38.5% of CNVs were intragenic. Tailored molecular testing approaches for difficult-to-sequence genes contributed to 12.9% (36/279) of diagnoses (confirmed with orthogonal methods).

Conclusions: These results emphasise the value of tailored approaches for difficult-to-sequence genes as well as the importance of including high-resolution CNV detection in enhancing the clinical utility of genetic testing using NGS panels for HHL.

L. Sarantaus: A. Employment (full or part-time); Significant; Blueprint Genetics. **K. Gall:** A. Employment (full or part-time); Significant; Blueprint Genetics Inc. **S. Tuupanen:** A. Employment (full or part-time); Significant; Blueprint Genetics. **M. Gandia:** A. Employment (full or part-time); Significant; Blueprint Genetics. **H. Duzkale:** A. Employment (full or part-time); Significant; Blueprint Genetics. **I. Saarinen:** A. Employment (full or part-time); Significant; Blueprint Genetics. **J. Sistonen:** A. Employment (full or part-time); Significant; Blueprint Genetics. **J. Koskenvuo:** A. Employment (full or part-time); Significant; Blueprint Genetics. **T. Alastalo:** A. Employment (full or part-time); Significant; Blueprint Genetics Inc.

P02.048.A North Carlina macular dystrophy: phenotypic variability and computational analysis of disease-implicated non-coding changes

David J. Green[1], *Eva Lenassi*[1], *Cerys S. Manning*[1], *David McGaughey*[2], *Vinod Sharma*[1], *Graeme C. Black*[1], *Jamie M. Ellingford*[1], *Panagiotis I. Sergouniotis*[1]

[1]*University of Manchester, Manchester, United Kingdom,* [2]*National Eye Institute, Bethesda, MD, USA.*

Introduction: North Carolina macular dystrophy (NCMD) is an autosomal dominant, congenital disorder affecting the central retina. Here, we report clinical and genetic findings in three families segregating NCMD and use epigenomic datasets from human tissues to gain insights into the effect of NCMD-implicated variants.

Materials and Methods: Clinical assessment and genetic testing were performed. Publicly-available transcriptomic and epigenomic datasets were analyzed and the 'Activity-by-Contact' (ABC) method for scoring enhancer elements and linking them to target genes was used.

Results: A previously-described, heterozygous, non-coding variant upstream of the *PRDM13* gene was detected in all six affected study participants (chr6:100,040,987G>C [GRCh37/hg19]). Inter- and intra-familial variability were observed; the visual acuity ranged from 0.0 to 1.6 LogMAR and fundoscopic findings ranged from visually insignificant, confluent, drusen-like macular deposits to coloboma-like macular lesions. Variable degrees of peripheral retinal spots (that were easily detected on widefield retinal imaging) were observed in all study subjects. Notably, a 6-year-old patient developed choroidal neovascularization and required treatment with intravitreal bevacizumab injections. Computational analysis of the five single nucleotide variants that are known to cause NCMD revealed that these non-coding changes lie within two putative enhancer elementspredicted to interact with *PRDM13* in the developing human retina. *PRDM13* was found to be expressed in the fetal retina, with highest expression in the amacrine precursor cell population.

Conclusions: We highlight the utility of widefield retinal imaging in individuals suspected to have NCMD and provide further evidence supporting the role of *PRDM13* dysregulation in the pathogenesis of this condition.

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P02.049.B Genotype phenotype correlations of 37 DFNB9 patients with auditory neuropathy and 17 new *OTOF* pathogenic variants

Sophie Achard[1], *Margaux SEREY-GAUT*[2], ***Laurence Jonard***[3], *Isabelle Rouillon*[1], *Marine Parodi*[1], *Natalie Loundon*[1], *Elisa Rubinato*[2], *Sandrine Marlin*[2]

[1]*Hopital Necker - Service d'ORL, Paris, France,* [2]*Hopital Necker - Service de Génétique Clinique, Paris, France,* [3]*Hopital Necker - Service de Génétique Moléculaire, Paris, France.*

Auditory neuropathy represents 5-10% of child's hearing loss. Pathogenic bi-allelic variations of *OTOF* result in autosomal recessive deafness DFNB9. We retrospectively studied the genotype-phenotype correlations of 37 cases from 30 families with pathogenic bi-allelic *OTOF* variations. Seventeen new pathogenic variants were identified. All patients had isolated auditory neuropathy. Hearing loss was pre-lingual in 78% of cases and profound in 70%. Hearing loss was progressive in 30%, fluctuating in 30% and temperature-sensitive in 22%. The diagnosis of auditory neuropathy was mainly based on the discordance of electrophysiological tests with acoustic otoemissions present (78%) and brainstem auditory evoked responses absent or desynchronized (81%). All patients with homozygous or compound heterozygous "loss of function" variants had congenital bilateral profound hearing loss, patients compound heterozygous for a "loss of function" variant and a missense variant had variable presentations. Those with two missense variants had a mild to severe hearing loss, which could be of secondary onset. 54% received cochlear implant rehabilitation, 16% of which were bilateral. Our study confirms a successful hearing rehabilitation with cochlear implants, with open word perception increasing from 0% before surgery to 80% at 8 years after implantation. However, cochlear implantation cannot be considered as a treatment (disturbance in noise, social difficulties and professional integration...). Gene therapy trials in mutant *OTOF* -/- adult mice have shown prolonged hearing rehabilitation, making it possible to consider a short-term therapeutic trial for this isolated congenital form of deafness (RHU AUDINNOVE 2019). This phenotype-genotype study is an essential prerequisite for the future therapeutic trial.

S. Achard: None. **M. Serey-gaut:** None. **L. Jonard:** None. **I. Rouillon:** None. **M. Parodi:** None. **N. Loundon:** None. **E. Rubinato:** None. **S. Marlin:** None.

P02.050.C A newborn with corneal clouding and a diagnosis of posterior polymorphous corneal dystrophy type 1 due to a duplication of the OVOL2 gene

Hester Y. Kroes[1], *M B. Muijzer*[1], *L Haer-Wigman*[2], *E S. M. Voskuil-Kerkhof*[1], *P M. van Hasselt*[1], *R P. L. Wisse*[1]

[1]*University Medical Centre Utrecht, Utrecht, Netherlands,* [2]*Radboud University Medical Centre, Nijmegen, Netherlands.*

Introduction: PPCD1 was recently shown to result from activating mutations in the promotor region of *OVOL2*. We present a new pathogenic mechanism in line with this observation. **Case report** An infant boy presented with progressive corneal clouding at the Department of Ophthalmology. Pregnancy was unremarkable, family history negative. Ophthalmologic investigation revealed bilateral diffuse corneal clouding and otherwise normal results. Physical examination was normal. A diagnosis of autosomal recessive CHED (congenital hereditary endothelial dystrophy) was suspected.

Methods: High throughput DNA-analysis was performed in the index case and his parents. An in silico panel analysis of 424 genes associated with visual disorders was performed consisting of analysis of single nucleotide variants and copy number variants (CNVs), followed by SNP-array.

Results: No causative single nucleotide variants were detected in *SLC4A11*, *OVOL2* or any other gene. CNV analysis and SNP array revealed a duplication of ~49 Kb in the chromosomal region 20p11.23 encompassing *OVOL2*, leading to a diagnosis of PPCD1. The duplication was absent in DNA from the parents.

Conclusion: We present the fifth index case of molecularly proven PPCD1. The underlying molecular abnormality, a duplication of the entire OVOL2 gene, suggests a gene dosage effect. So far, four PPCD1 families have been described, each with an activating variant in a distinct part of the promotor region of *OVOL2*. OVOL2 is a transcription factor acting as a repressor of *ZEB1*. Loss of function variants in *ZEB1* cause PPCD3. This case seems to confirm that gain of function of *OVOL2* causes PPCD1.

H.Y. Kroes: None. **M.B. Muijzer:** None. **L. Haer-Wigman:** None. **E.S.M. Voskuil-Kerkhof:** None. **P.M. van Hasselt:** None. **R.P.L. Wisse:** None.

P02.051.D Functional characterization of non-canonical splice variants in aniridia by minigenes and ex-vivo approaches

Alejandra Tamayo[1,2], *María Tarilonte*[1], *Jennifer Moya*[1], *Patricia Ramos*[1], *Saoud T Swafiri*[1,2], *Fiona Blanco*[1,2], *Cristina Villaverde*[1,2], *Carmen Ayuso*[1,2], *Marta Cortón*[1,2]

[1]*Instituto de Investigación Sanitaria Fundación Jiménez Díaz University Hospital, Madrid, Spain,* [2]*Centre for Biomedical Network Research on Rare Diseases (CIBERER), Madrid, Spain.*

Introduction: Mutations in *PAX6* cause aniridia, a congenital disorder characterized by several structural eye anomalies. We describe the development of in-vitro and ex-vivo tools for the functional characterization of potentially spliceogenic variants and their impact on the canonical *PAX6* splicing.

Materials and Methods: For minigene assays, a genomic segment encompassing the region of interest of *PAX6* was cloned into expression vectors and variants were introduced by site-directed mutagenesis. In-vitro assays were performed by transient transfection into HEK293 and/or ARPE19 cell lines. Lymphoblastoid cell lines (LCL) were established by Epstein Barr virus-mediated transformation of blood lymphocytes from patients carrying splicing variants and control individuals. Total RNA was extracted and reversely transcribed. The splicing patterns of mRNA transcripts were compared by semiquantitative PCR and sequencing.

Results: We developed four minigene *PAX6* constructs: i) exons 1 to 4, ii) exons 5a and 6, iii) exons 5 to 7 and iv) exons 8 to 11, respectively. To date, a total of 13 potentially spliceogenic variants were assayed and 11 of them showed aberrant splicing patterns. Expression analysis in 4 patient-derived LCL showed alterations on splicing patterns. Two out these 4 variants were also tested by minigene assays and the findings on splicing patterns showed a correlation between both approaches.

Conclusions: Minigene assays carrying multiple exons and LCL studies are useful strategies to gain insight into the pathogenicity of non-canonical splice *PAX6* variants. These methods are easy-to-carry approaches for robust splice variant analysis and to help bring molecular diagnosis to aniridia patients.

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P02.052.A Clinical molecular genetic age characteristics of congenital aniridia in children

Natella Sukhanova[1], *Rena Zinchenko*[2], *L.A.Katargina.A.V.Marakhonov,T.A.Vasilieva,*

[1]*Federal State Budgetary Institution of Health's Central Clinical Hospital of the Russian Academy of Sciences, Moscow, Russian Federation,* [2]*Federal State Budgetary Scientific Institution "Research Centre for Medical Genetics, Moscow, Russian Federation.*

Introduction: The relationship between the clinical features of the phenotype and the genotype confirmed by molecular genetic methods in children with aniridia expands our understanding of the clinical course of the disease and corrects possible treatment. Congenital aniridia (OMIM # 106210) is a monogenic hereditary pathology. The leading diagnostic signs are congenital absence of iris tissue, hypoplasia of fovea, accompanied by nystagmus.

Materials and Methods: The data of ophthalmological examination and molecular genetic analysis of 83 patients from 0 to 18 years old were analyzed. Mutations were previously identified in 56 patients as a result of sequencing of exons of the PAX6 gene. In 27 patients, various deletions of the 11p13 region were identified by MLPA.

Results: According to clinical signs, patients with congenital aniridia were distributed: the presence of complete or partial aniridia, nystagmus, keratopathy, cataracts and glaucoma. With nonsense mutations, the complete absence of iris tissue and the development of keratopathy observed in average age 5 ± 1 years. Patients with open reading frame shift mutations are significantly more likely to develop complicated cataract and glaucoma in average age 10 ± 2 years. Partial aniridia occurs with missense mutations significantly more often.

Conclusions: The identification of age-related characteristics depending on the detected mutations is of not only clinical, but also scientific interest, since it indicates one of the mechanisms of regulation of the PAX6 gene function in case of damage to the organ of vision.The research was partially supported by RSF grant №17-15-01051

N. Sukhanova: None. **R. Zinchenko:** None.

P02.053.B Minigene expression system for assess the effect of variants in the *PAX6* gene on pre-mRNA splicing

Ksenia Davydenko, *Alexandra Filatova, Mikhail Skoblov*

Research Centre for Medical Genetics, Moscow, Russian Federation.

Introduction: Mutations in the *PAX6* gene affect the normal development of the eye and lead to a spectrum of phenotypes, the most severe is aniridia. Today more than 600 mutations in *PAX6* have been reported, about 100 of them were annotated as affecting splicing. Moreover, variants affecting splicing could masquerade as missense, nonsense or synonymous in databases. In order to establish the effect of reported mutations on splicing, a functional analysis is required.

Materials and methods: Bioinformatics analysis was performed using the SpliceAI (Illumina, USA) and MaxEntScan (MIT, USA) tools. The minigene expression system was used to evaluate the functional effect of SNVs on splicing.

Results: We created a system of 8 plasmids containing all 10 coding exons of the *PAX6* gene. Plasmids were tested for correct splicing and resulted in normally spliced wild-type transcripts. For all 354 *PAX6* SNVs from the HGMD, LOVD, and ClinVar databases we predicted the probability of influence on splicing. We selected 18 intronic SNVs outside ±1,2 position whose effect on splicing has not been confirmed experimentally and 20 exonic (synonymous, missense and nonsense) SNVs which actually could affect splicing. Functional analysis of these SNVs in the minigene expression system revealed a wide range of splicing defects.

Conclusion: We created and successfully tested a minigene expression system for assess the effect of variants in *PAX6* gene on pre-mRNA splicing. This system can be used to establish the pathogenicity of splicing variants in the *PAX6* gene, and to reclassify misclassified variants located in databases.

K. Davydenko: None. **A. Filatova:** None. **M. Skoblov:** None.

P02.054.C novel phenotype-genotype correlation with *PEX6* gene in Saudi patients with heimler syndrome

Basamat Almoallem

Department of Ophthalmology, King Saud University, Riyadh, Saudi Arabia, Riyadh, Saudi Arabia.

Purpose: Peroxisome biogenesis disorders (PBDs; MIM# 601539) are heterogeneous disorders caused by defects in genes encoding proteins that are essential for peroxisomal matrix and membrane

proteins. Heimler syndrome is one of the PBDs that is caused by mutations in PEX1 and PEX6 genes. In this study, we aimed to fully characterise the clinical and molecular aspects of two Saudi probands who were diagnosed early with Usher syndrome.

Method: We set up a comprehensive clinical and molecular genetic workflow including detailed ophthalmological and systemic assessments followed by genome-wide SNP microarrays analysis and targeted PEX6 gene screening by Sanger sequencing.

Results: Clinically: both probands appeared dysmorphic with long faces, high forehead, short nose, small low set ears, and full lips. Moreover, advanced inherited retinal dysfunction represented by waxy pallor optic disc, attenuated vessels, RPE mottling with intraretinal bony spicules pigmentations and central foveal atrophic changes in both eyes with bilateral sensory neural hearing loss and amelogenesis imperfecta. Genetically: an autozygous block was identified on chromosome 6p21.1 encompassing PEX6 gene using SNP microarrays. Novel PEX6 (NM_000287.3)c.290T>G (p.Val97Gly) was found and co-segregated with the phenotype in both families and found to be "likely pathogenic" according to ACMG guidelines.

Conclusion: Our study represents the first report of PEX6 associated Heimler syndrome in the middle east and considered to be the third in the literature so far. It highlights the importance of combining molecular diagnosis with the clinical findings to expand our knowledge of PEX6-related PBDs phenotype and the allelic spectrum for this gene.

B. Almoallem: None.

P02.055.D Investigation of transcriptional dysregulation events driving Posterior Polymorphous Corneal Dystrophy type 1

Nathaniel J. Hafford-Tear[1], *Lubica Dudakova*[2], *Stephen J. Tuft*[1,3], *Amanda Sadan*[1], *Nihar Bhattacharyya*[1], *Christina Zarouchlioti*[1], *Alison J. Hardcastle*[1], *Petra Liskova*[2,4], *Alice E. Davidson*[1]

[1]*UCL Institute of Ophthalmology, London, United Kingdom,* [2]*Research Unit for Rare Diseases, Department of Pediatrics and Inherited Metabolic Diseases, First Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic,* [3]*Moorfields Eye Hospital, London, United Kingdom,* [4]*Department of Ophthalmology, First Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic.*

Purpose: Posterior Polymorphous Corneal Dystrophy (PPCD) is associated with a dysregulated cell state, induced by mutations that alter the levels of epithelial mesenchymal transition (EMT)-regulating transcription factors ZEB1, GRHL2 or OVOL2. Here we investigate the transcriptomic signature of dysregulation in PPCD type 1 (PPCD1) corneal endothelial cells (CECs) to identify biomarkers of the disease as potential therapeutic targets.

Methods: Primary CECs were cultured from five individuals affected with PPCD1 and five controls. All PPCD1 individuals were confirmed to harbour the same regulatory mutation in the *OVOL2* promoter (NM_021220:c.−370T>C). Total RNA was extracted from each primary culture and paired-end Illumina RNA sequencing was performed. Transcriptomic analysis was performed using DESeq2 and IsoformSwitchAnalyzeR programs to investigate differential gene expression and splicing events in PPCD1 CECs compared to control CECs.

Results: Utilizing the EMT Gene Database, 279 EMT-associated genes were found to be highly dysregulated (p-value < .05; Log-2 fold change >1) in PPCD1 including *CDH1*, *OVOL2*, *GRHL2*, *GATA6* and the epithelial splice regulator *ESRP1*. Alternative splicing in PPCD1 vs controls was further identified for ESRP1 targets, *CD44* and *FGFR2*.

Conclusions: Our study detected aberrant upregulation of *OVOL2* in PPCD1, supporting the hypothesis for pathogenic mechanism, and identified other EMT-associated genes and pathways that are significantly disrupted in PPCD1 CECs. Our data suggests that overexpression of the epithelial cell type-specific splicing regulator, *ESRP1*, induces aberrant splicing of *CD44* and *FGFR2*. We hypothesise that this mechanism contributes to the 'epithelialisation' of the corneal endothelium observed in PPCD1 and may represent a target for future therapeutic interventions.

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P02.056.A Clinical-genetic correlation of the functional state of the retina in ABCA4-associated pathology

Vitaly V. Kadyshev[1], *Andrei V. Marakhonov*[1], *Inna V. Zolnikova*[1,2], *Sergey I. Kutsev*[1], *Rena A. Zinchenko*[1]

[1]*Research Centre for Medical Genetics, Moscow, Russian Federation,* [2]*Helmholtz National Medical Research Center of Eye Diseases, Moscow, Russian Federation.*

Aim: To set clinical and genetic correlations of retinal pathology by ABCA4 gene mutations, considering the functional state **Material and methods:** 15 Russian patients aged from 7 to 32 y.o. with inherited eye diseases *ABCA4*-associated. All patients besides ophthalmic examination, spectral-OCT, fundus autofluorescence, full-field electroretinogram (ERG), 30-Hz flicker ERG, macular chromatic ERG (MERG) to red stimulus, molecular genetic studies included Next Generation Sequencing and Sanger direct sequencing.

Results: In *ABCA4*-associated Stargardt disease (SD) genotype [p.L541P, p.A1038V] of «frequent» mutations was revealed in 9 patients, in 2 cases in was associated another «frequent» mutation p.G1961E. In 4 patients with genotype [p.L541P, p.A1038V] «severe» phenotype of Stargardt disease was found: with large defect of the ellipsoid zone, severely subnormal macular ERG (MERG) to red stimulus and subnormal 30 Hz flicker and full-field maximal ERG. In 2 cases with genotype [p.L541P, p.A1038V] and mutation p.G1961E was found «mild» phenotype. Nonrecordable MERG was found in 7 of 15 cases, 8 patients had subnormal. Subnormal 30 Hz flicker ERG was found in 8 patients with SD. Maximal ERG was reduced in 6 patients with SD.

Conclusions: The study made it possible to assess the effect of mutations in the ABCA4 gene not only on the structural changes but also on the functional state of the retina. The study expands the range of clinical and genetic features of hereditary ophthalmological pathology associated with the ABCA4 gene. Supported by RFBR (project № 19-015-00122 A), within the state task of the Ministry of education and science of Russia

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P02.057.D Health-related quality of life amongst patients with retinal dystrophies

Deborah Schofield[1], ***Joshua Kraindler***[1], *Rupendra Shrestha*[1], *Sarah West*[1], *Owen Tan*[1], *Alan Ma*[2,3], *Diana Jelovic*[2], *John Grigg*[4,3,5], *Robyn Jamieson*[2,4,3]

[1]*GenIMPACT: Centre for Economic Impacts of Genomic Medicine, Macquarie Business School, Macquarie University, Sydney, Australia,* [2]*Eye Genetics Research Unit, Children's Medical Research Institute, University of Sydney, Sydney, Australia,* [3]*Sydney Children's Hospitals*

Network, Sydney, Australia, [4]Save Sight Institute, Sydney, Australia, [5]Sydney Eye Hospital, Sydney, Australia.

Introduction: Retinal dystrophies (RD) are diseases leading to incurable blindness attributable to variations in more than 200 genes. There have been significant advances in gene therapies including Voretigene Neparvovec (VN), which was recently approved for intervention in RD due to variants in *RPE65*, by the Food and Drug Administration, and the National Health Service. Approval of publicly funded interventions often require cost-effectiveness analysis (quality adjusted life years (QALYs) gain). To our best knowledge, studies reporting health-related utility of RD - a key metric in computing QALYs - are scarce or have insufficient or indirect measures of QoL outcomes. We aim to present the first paper examining the health-related utility of RD using an established QoL measure - the Assessment of Quality of Life-8D (AQoL-8D).

Materials and methods: As part of the EPIC-Vision study, we collected patient (age≥18) reported AQoL-8D data as part of a customised survey. AQoL-8D measures functioning across eight dimensions: independent living, pain, senses, mental health, happiness, coping, relationships, self-worth. The EPIC-Vision survey also collected data on visual functioning (NEI-VFQ), and socioeconomic data.

Results: Average health-related utility for patients with RD was 0.6, significantly lower than the average population norm (0.8). Patient's scores were relatively lower on independent living, mental health, relationship, self-worth, and senses. Regression analysis showed that patients with better visual functioning score had better health-related utility.

Conclusions: We reported for the first time, health-related utility for RD patients, which can be used to inform further studies on new interventions.Funding: NHMRC Partnership Grant (APP1116360).

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P02.058.C Genetic analysis of patients with retinal dystrophies in Croatia

Adriana Bobinec[1,2], *Mijana Kero*[1,2], *Ljubica Boban*[1,2], *Nikolina Vidan Rogulj*[1], *Ana-Maria Meašić*[1,2], *Ivona Sansović*[1,2], *Leona Morožin Pohovski*[1], *Mirjana Bjeloš*[3,4], *Mladen Bušić*[3,4], *Ingeborg Barišić*[1,2]

[1]*Children's Hospital Zagreb, Zagreb, Croatia,* [2]*Department of Medical Genetics and Reproductive Health, Children's Hospital Zagreb, Scientific Centre of Excellence for Reproductive and Regenerative Medicine (CERRM), University of Zagreb School of Medicine, Zagreb, Croatia,* [3]*University Department of Ophthalmology, University Clinical Hospital Sveti Duh, Zagreb, Croatia,* [4]*Faculty of Dental Medicine and Health, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia.*

Introduction: Retinal dystrophies (RD) are a group of inherited retinal disorders characterized by progressive photoreceptors and pigment epithelial cells dysfunction causing severe visual loss and eventual blindness. Recently augmentation gene treatment has been approved for patients with biallelic mutations in the *RPE65* gene known to cause pigmentary retinopathy type 20 and Leber congenital amaurosis 2. So far gene therapy in these patients provided improvement of functional vision without serious adverse reactions raising great interest for genetic testing of RD.

Materials and methods: Next-generation sequencing (NGS) was performed in 60 patients with a referral diagnosis of retinal dystrophy using Illumina TruSight One Kit.

Results: In 36 (60) patients different pathogenic variants were found in *RP1*, *RP2, RHO, EYS, USH2A, BEST1, PROM1, C9, ABCA4, ADGRV1, BEST1, CEP290* genes. In two patients identified variants were classified as variants of unknown significance. In 22 patients NGS analysis did not reveal mutations in genes associated with RD.

Conclusion: Molecular genetic testing of retinal dystrophies has been successfully used to clarify clinical diagnoses and direct genetic counselling. NGS proved to be efficient and useful method for setting up the diagnosis in 63% of patients.This study was supported by CERRM, Republic of Croatia, and by the EU through ERDF, under grant agreement No. KK.01.1.1.01.0008, project „Reproductive and Regenerative Medicine - Exploring New Platforms and Potentials".

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P02.059.D Implementation of the targeted retinal dystrophy panel in the routine genetic diagnostics of retinal disorders in Polish patients

Ewa Matczyńska[1,2], *Przemysław Łyszkiewicz*[1], *Anna Wąsowska*[1,2], *Robert Szymańczak*[1], *Ewa Suchecka*[1], *Katarzyna Stradomska*[1], *Marta Beć*[1], *Maria Jędrzejowska*[1], *Sławomir Teper*[2], *Maciej Krawczyński*[3], *Anna Boguszewska-Chachulska*[1]

[1]*Genomed S.A., Warsaw, Poland,* [2]*Chair and Clinical Dept. of Ophthalmology, Medical University of Silesia, Katowice, Poland,* [3]*Center of Medical Genetics Genesis Sp. z o.o., Poznań, Poland.*

A targeted panel approach for genetic diagnosis of inherited retinal dystrophies (IRD) was developed, based on the results of the preceding NeuStemGen project (a cohort of Polish IRD patients analysed using WES). The implementation of the approach in routine diagnostics provided an opportunity to assess its performance along with strengths and weaknesses.

Polish patients with clinical symptoms of retinal dystrophies (N = 168) were subjected to NGS using a custom panel capturing coding regions of 270 IRD genes (RetNet), developed with the Roche NimbleGen SeqCap EZ. Bioinformatic analysis was performed with the GATKv4 and CoNVaDING (for CNV analysis), using the GRCh38 reference genome. Variant analysis and interpretation were completed according to ACMG guidelines using the in-house variant interpretation tool - BroVar.

Overall sequencing data metrics presented a satisfactory level of target coverage (mean coverage - 255x ± 55; fraction of target bases covered >20x - 0.996 ± 0.001) and high heterozygote SNP calling sensitivity (0.998 ± 0.0003), indicating near-optimal performance for SNPs and small indels, providing diagnosis confirmation for 77 patients. CNV analysis enabled finding causative variants for additional 9 patients (51% overall), however, CNV identification performed sub-optimal in several batches due to technical bias and a low number of samples per pool.

The targeted retinal panel approach has been successfully applied in routine IRD diagnostics of Polish patients, expanding the knowledge of IRD genetic background in the Polish population, simultaneously allowing for a cost-effective analysis (comparing to WES). Despite reliable coverage, identification of CNVs remains challenging. Further refinement of CNV and more complex variants' calling is necessary.

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P02.061.B Metastasis suppressor 1 <*MTSS1*> as a novel candidate gene for inherited retinal dystrophy <IRD>

Solomon Merepa[1], *Suzanne Broadgate*[1], *Jing Yu*[1], *Sumathi Sekaran*[1], *Susan Downes*[1,2], *Stephanie Halford*[1], *United Kingdom Inherited Retinal Dystrophy Consortium (UKIRDC)*

[1]*Nuffield Laboratory of Ophthalmology, Department of Clinical Neuroscience, University of Oxford, Oxford, United Kingdom,* [2]*Oxford Eye Hospital, John Radcliffe Hospital, Oxford University Hospitals NHS Foundation Trust, Oxford, United Kingdom.*

Introduction: Over 260 genes underlying monogenic forms of inherited retinal dystrophies (IRDs) have been identified, enabling the development of treatment regimens such as gene therapy. However, there are still IRD cases whose underlying genetic causes are yet to be identified, prompting the need for continued disease-gene identification studies.

Methods: Two affected members of a family diagnosed with dominant retinitis pigmentosa (adRP) underwent whole-exome sequencing (WES). Various bioinformatics tools and gene sequencing databases including CADD and gnomAD were used to annotate and prioritise candidate variants. Segregation analysis was performed using Sanger sequencing. A variant segregating with the condition was characterised for expression, localisation and function in the mammalian retina using techniques including PCR, western immunoblotting, immunostaining, and site-directed mutagenesis.

Results: Of 22 shared dominant variants determined from WES, a mutation in the *MTSS1* gene (c.624_628del (p.E213del)) segregated with disease in the family. The expression and function of MTSS1 in the mammalian retina has not been previously described. We report, for the first time, expression of *MTSS1* in the human retina and using antibodies specific for Mtss1, we demonstrate immunostaining in layers of the mouse retina including the photoreceptors. Using mutant expression constructs, we are currently investigating if the mutation affects localisation and function of MTSS1 at the cellular level.

Conclusions: We provide here, for the first time, preliminary evidence linking mutation in the *MTSS1* with dominant RP. Elucidating the specific role of MTSS1 in the mammalian retina could potentially inform therapeutic approaches for IRDs.

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P02.062.C First systematic molecular genetic analysis using NGS analysis of 120 Greek patients with retinal dystrophy

Smaragda Kamakari[1], *Stavrenia Koukoula*[2], *Vasiliki Kokkinou*[1], *Lonnecke Haer-Wigman*[3], *Ioannis Datseris*[4], *Miltiadis Tsilimbaris*[5]

[1]*Ophthalmic Genetics Unit, Athens, Greece,* [2]*Ophthalmica Institute of Ophthalmology and Microsurgery, Thessaloniki, Greece,* [3]*Department of Human Genetics, Donders Centre for Neuroscience, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands,* [4]*Department of Retinal Disorders, OMMA, Ophthalmological Institute of Athens, Athens, Greece,* [5]*Department of Ophthalmology, University of Crete School of Medicine, Heraklion, Greece.*

Purpose: Inherited Retinal Dystrophies (IRDs) are characterized by clinical variability and genetic heterogeneity. The aim of this study was to molecularly diagnose 120 Greek patients with different forms of IRDs.

Materials and Methods: 120 unrelated Greek patients were analyzed by Next Generation Sequencing (NGS), 13 and 107 of them using a 105 retinal and a 287 ophthalmic gene panel, respectively as described (Ellingford JM et al. J Med Genet 2016, Haer-Wigman L et al. Eur J Hum Genet. 2017). Additional analysis methods were used (Sanger, MLPA, array-CGH) in 6 cases.

Results: Potentially pathogenic mutations were detected in 47 retinal dystrophy genes including ABCA4, PRPF31, SPATA7, MERTK, FAM161A, CDHR1, USH2A, CNGB1, PROM1, RPGR and RP2 genes. The detection mutation rates were 46.15% (6/13) and 79.4% (85/107) for the 105 and 287 gene panels, respectively. These mutation rates were achieved using complementary methods in 6 cases. Final diagnoses included retinitis pigmentosa, Usher syndrome, cone-rod dystrophy and Leber congenital amaurosis and two rare cases of Knobloch and Oliver-McFarlane syndromes due to mutations in the COL18A1 and PNPLA6 genes, respectively.

Conclusions: This is the first systematic investigation of the molecular identity of 120 Greek patients with various subforms of IRDs by NGS and complementary methods leading to an overall mutation rate of 75.8%. A plethora of novel mutations was documented further expanding the genetic heterogeneity. The molecular identification established the complete diagnosis of the patients thus contributing to family making decision, prognosis and candidacy to current and future treatments.

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P02.064.A Whole locus sequencing identifies a prevalent founder deep intronic *RPGRIP1* pathologic variant in the French Leber congenital amaurosis cohort

Isabelle Perrault[1], *Sylvain Hanein*[2], *Xavier Gérard*[1], *Nelson Mounguengue*[1], *Ryme Bouyakoub*[1], *Mohammed Zarhate*[3], *Cécile Fourrage*[4], *Fabienne Jabot-Hanin*[4], *Béatrice Bocquet*[5], *Isabelle Meunier*[5], *Xavier Zanlonghi*[6], *Josseline Kaplan*[1], *Jean-Michel Rozet*[1]

[1]*Laboratory of Genetics in Ophthalmology (LGO), INSERM UMR1163, Institute of Genetic Diseases, Imagine and Paris Descartes University, Paris, France,* [2]*Translational Genetics, Institute of Genetic Diseases, Imagine and Paris Descartes University, INSERM UMR1163, PARIS, France,* [3]*Genomics Platform, Institute of Genetic Diseases, Imagine and Paris Descartes University, Paris, France,* [4]*Bioinformatic Platform. Institute of Genetic Diseases, Imagine and Paris Descartes University, Paris, France,* [5]*Centre de Référence des Affections Sensorielles Génétiques, Institut des Neurosciences de Montpellier, CHU-Saint Eloi Montpellier, Montpellier, France,* [6]*Eye Clinic Jules Verne, Nantes, France.*

Leber congenital amaurosis (LCA) encompasses the earliest and most severe retinal dystrophies and can occur as a non-syndromic or a syndromic disease. Molecular diagnosis in LCA is of particular importance in clinical decision-making and patient care since it can provide ocular and extraocular prognostics and identify patients eligible to developing gene-specific therapies. Routine high-throughput molecular testing in LCA yields 70%-80% of genetic diagnosis. In this study, we aimed to investigate the non-coding regions of one non-syndromic LCA gene, *RPGRIP1*, in a series of six families displaying one single disease allele after a gene-panel screen of 722 LCA families which identified 26 biallelic *RPGRIP1* families. Using trio-based high-throughput whole locus sequencing (WLS) for second disease alleles, we identified a founder deep intronic mutation (NM_020366.3:c.1468-128T>G) in 3/6 families. We employed Sanger sequencing to search for the pathologic variant in unresolved LCA cases (106/722) and identified three additional families (2 homozygous and 1 compound heterozygous with the c.930+77A>G deep intronic

change). This makes the c.1468-128T>G the most frequent *RPGRIP1* disease allele (8/60, 13%) in our cohort. Studying patient lymphoblasts, we show that the pathologic variant creates a donor splice-site and leads to the insertion of the pseudo-exon in the mRNA, which we were able to hamper using splice-switching antisense oligonucleotides (AONs), paving the way to therapies. This work was supported by grants from Retina France, UNADEV-AVIESAN ITMO MNP (R16073KS) and VISIO; J.-M.R. is member of ERN-EYE project (739534-'ERN-EYE).

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P02.065.B Genetic study of Italian families affected by small fibre neuropathy identified variants in predisposing pain phenotype

Kaalindi Misra[1], *Silvia Santoro*[1], *Andrea Zauli*[1], *Margherita Marchi*[2], *Erika Salvi*[2], *Raffaella Lombardi*[2], *Daniele Cazzato*[2], *Filippo Martinelli Boneschi*[3,4], *Massimo Filippi*[5,6,7,8], *Federica Esposito*[1,5], *Giuseppe Pinter Lauria*[2,9]

[1]*Laboratory of Human Genetics of Neurological Disorders, IRCCS San Raffaele Scientific Institute, Milan, Italy,* [2]*Neuroalgology Unit, Foundation IRCCS Carlo Besta Neurological Institute, Milan, Italy,* [3]*Department of Biomedical Sciences for Health, University of Milan, Milan, Italy,* [4]*Neurology Unit, Foundation IRCCS Cà Granda Ospedale Maggiore Policlinico, Milan, Italy,* [5]*Neurology and Neurorehabilitation Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy,* [6]*Vita-Salute San Raffaele University, Milano, Italy,* [7]*Neurophysiology Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy,* [8]*Neuroimaging Research Unit, Institute of Experimental Neurology, Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Milan, Italy,* [9]*Department of Biomedical and Clinical Sciences "Luigi Sacco", University of Milan, Milan, Italy.*

Peripheral Neuropathy (PN) affects 2.4% of people and almost 50% of general population is known to have pain-related symptoms. Genetic studies in painful PN (PPN) revealed that Voltage Gated Sodium Channels (VGSCs) genes are involved in pain amplification. Here we aimed to broaden the genetic aspect of PPN by using whole exome sequencing (WES).

Six families with PPN were selected having at least one affected member, positive neurological examination and pain questionnaire result with numerical rating score >=4. Variants were filtered with manually curated gene panel, allele frequency (AF) and computational predictors. Segregation causative/protective models were applied according to pedigree and sharing models were applied after grouping probands of each family.

According to segregation causative and protective model, we found 129 and 112 variants respectively (AF<=10%) across families. Among genes shared between two families with causative approach, variants were observed in *SCN9A*, *SV2C* and *DST*, whereas protective variants in *TRPM2* and *LRP1*. In shared model, we identified 21 variants and 53 genes shared across >=3 probands. Among shared genes with predicted high-impact variants in probands were observed in *SCN9A, SCN7A, P2RY4, P2RX7, TRPV4* and *TRPM1*.

WES approach appears powerful in mutation detection and in revealing new genotype-phenotype association. In addition to VGSCs, other gene families including Transient Receptor Potential and Purinergic Receptor seem to play a role in pain modulation. The same approach will be replicated on new families already sequenced before proceeding with ad hoc functional experiments to deepen the role of genes in painful phenotype.

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P02.066.C Human knockouts of olfactory receptors genes and smell perception impairment in a large Italian cohort

Paola Tesolin[1], *Maria Pina Concas*[2], *Massimiliano Cocca*[2], *Margherita Francescatto*[1], *Agnese Luglio*[1], *Beatrice Spedicati*[1], *Agense Feresin*[1], *Anna Morgan*[2], *Paolo Gasparini*[2,1], *Giorgia Girotto*[2,1]

[1]*University of Trieste, Trieste, Italy,* [2]*Institute for Maternal and Child Health – IRCCS "Burlo Garofolo", Trieste, Italy.*

Genetic variations across Olfactory Receptors (OR) genes influence the diversity of odorant sensitivity among individuals. Nevertheless, there is still a lack of knowledge regarding the genetic bases of smell performance. Whole-genome sequencing and smell phenotypes data of 218 Italian individuals allowed the identification of 41 natural OR knockouts (KO) (i.e., genes carrying biallelic loss of function variants). In detail, the following steps have been performed:1. recursive partitioning analysis to evaluate the effect of OR-KO genes' burden on the smell perception (measured by the number of errors in the Sniffin Sticks test),2. evaluation of the expression of these genes in human and mouse tissues using publicly available data,3. estimation of the presence of organ-related diseases in Human KO (HKO) individuals for OR expressed in non-olfactory tissues (Fisher test). Regarding (1), ageing and the burden of OR-KO led to a worsening of smell perception (p-value <0.05). In particular, the effect of the OR-KO burden was higher in younger individuals (aged≤57). With respect to (2), 33/41 OR genes have been detected in the human olfactory system (OS) and 27 in other tissues, while among the 60 putative ORs mouse homologues, 58 were expressed in the OS and 37 in other tissues. Finally, (3) 14 pathologies resulted in being more frequent in OR-HKO individuals (p-value < 0.01). Our work confirms the predominant role of age in worsening smell perception and highlights, for the first time, the role of the burden of OR-KO genes.

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P02.067.D Insights into the pathogenesis of Stargardt disease associated with splicing mutation c.5714+5G&gtA in *ABCA4* gene

Jana Sajovic[1], *Andrej Meglič*[1], *Zelia Corradi*[2,3], *Mubeen Khan*[2,3,4], *Frans F.M. Cremers*[2,3], *Claire-Marie Dhaenens*[2,5], *Martina Jarc Vidmar*[1], *Damjan Glavač*[6], *Aleš Maver*[7], *Marija Volk*[7], *Borut Peterlin*[7], *Marko Hawlina*[1], *Ana Fakin*[1]

[1]*Eye Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia,* [2]*Department of Human Genetics, Radboud University Medical Center, Nijmegen, Netherlands,* [3]*Donders Institute for Brain, Cognition and Behaviour, Radboud University, Nijmegen, Netherlands,* [4]*Department of Pathology and Medical Biology, University Medical Center Groningen, Groningen, Netherlands,* [5]*Univ. Lille, Inserm, CHU Lille, U1172-LilNCog-Lille Neuroscience & Cognition, Lille, France,* [6]*Department of Molecular Genetics, Institute of Pathology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia,* [7]*Clinical Institute of Medical Genetics, University Medical Centre Ljubljana, Ljubljana, Slovenia.*

Introduction: >1200 *ABCA4* variants cause Stargardt disease making genotype-phenotype correlations difficult. Approximately 15% of Slovenian Stargardt patients harbour c.5714+5G>A which allowed phenotypic specification of this variant.

Methods: Sixteen patients with different genotypes were recruited. Group 1 harboured c.5714+5G>A in *trans* with a null mutation (N = 6) and group 2 had two null mutations (N = 10). Correlation between the degree of retinal pigment epithelium (RPE) atrophy (represented by the area of decreased autofluorescence on fundus imaging) and loss of photoreceptors (represented by outer nuclear layer (ONL) thickness on optical coherence tomography (OCT) and pattern electroretinography (PERG) amplitudes) was compared between groups. Effect of c.5714+5G>A on RNA splicing was analyzed using reverse transcription (RT)-PCR of mRNA isolated from patient-derived photoreceptor progenitor cells (PPCs).

Results: RT-PCR of mRNA from PPCs from a patient carrying c.4539+1G>T and c.5714+5G>A using primers in exons 38 and 44 showed a major normal product and minor exon 40 and exon 39/40 deletion products. Statistical analysis showed that for a similar RPE atrophy area, patients in Group 1 had significantly better structural (thicker ONL) and functional (higher PERG amplitudes) preservation of photoreceptors (multiple linear regression, $p < 0.01$). Preserved photoreceptors were seen above preserved RPE on OCT in Group 1.

Conclusions: Patients harbouring c.5714+5G>A exhibit distinctly different phenotype than patients carrying two null mutations, characterized by less photoreceptor loss at comparable degrees of RPE loss. We hypothesize that remaining ABCA4 function originating from the retained normally spliced product spares photoreceptors from early degeneration and shifts the pathogenesis to a primarily RPE disease.

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P02.068.A Biallelic pathogenic variants in COL9A3 confirm autosomal recessive stickler syndrome

Aboulfazl Rad[1], *Maryam Najafi*[2], *Fatemeh Suri*[3], *Stephen Loum*[1], *Ehsan Ghayoor Karimiani*[4], *David Murphy*[5], *Mohammad Doosti*[4], *Narsis Daftarian*[6], *Paria Najarzadeh Torbati*[4], *Afrooz Moghaddasi*[3], *Hamid Ahmadieh*[3], *Mohsen Rajati*[7], *Narges Hashemi*[8], *Barbara Vona*[1], *Miriam Schmidts*[9]

[1]*Department of Otorhinolaryngology, Head and Neck Surgery, Tübingen, Germany,* [2]*3Pediatric Genetics Divison, Center for Pediatrics and Adolescent Medicine, University Hospital Freiburg, Freiburg University Faculty of Medicine, Mathildenstrasse 1, 79106 Freiburg, Germany, Freiburg, Germany,* [3]*Ophthalmic Research Center, Research Institute for Ophthalmology and Vision Science, Shahid Beheshti University of Medical Sciences, Tehran, Iran, Tehran, Iran, Islamic Republic of,* [4]*Department of Molecular Genetics, Next Generation Genetic Polyclinic, Mashhad 009851, Iran, Mashhad, Iran, Islamic Republic of,* [5]*Department of Clinical and Movement Neurosciences, UCL Queen Square Institute of Neurology, University College London, United Kingdom, London, United Kingdom,* [6]*Ocular Tissue Engineering Research Center, Research Institute for Ophthalmology and Vision Science, Shahid Beheshti University of Medical Sciences, Tehran, Iran, Tehran, Iran, Islamic Republic of,* [7]*Associate professor of Otorhinolaryngology, Ghaem Hospital, Sinus and Surgical Endoscopic Research Center, Department of Otorhinolaryngology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran, Mashhad, Iran, Islamic Republic of,* [8]*Department of Pediatric Neurology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran, Mashhad, Iran, Islamic Republic of,* [9]*Pediatric Genetics Divison, Center for Pediatrics and Adolescent Medicine, University Hospital Freiburg, Freiburg University Faculty of Medicine, Mathildenstrasse 1, 79106 Freiburg, Germany, Freiburg, Germany.*

Introduction: Stickler syndrome (STL) is a clinically and molecularly heterogeneous connective tissue disorder that includes ocular impairment, hearing loss, joint and craniofacial abnormalities. Pathogenic variants occurring in a variety of genes cause STL, mainly inherited in an autosomal dominant fashion. Autosomal recessive STL is ultra-rare with only a few cases reported to date, including three families with biallelic *COL9A3* variants. Here, we report three unrelated families clinically diagnosed with STL who present novel biallelic loss of function variants in *COL9A3*.

Materials and Methods: Exome sequencing (ES) was employed to sequence the DNA of probands from three unrelated Iranian families. Conventional PCR and Sanger sequencing were performed to confirm segregation of the candidate variants in accessible family members.

Results: Our data revealed three novel loss of function variants in *COL9A3* in three unrelated families. All affected individuals shared moderate- to high-myopia and moderate to severe sensorineural hearing loss. The other clinical observations including hypermobility, mild spondyloepiphyseal dysplasia, midface hypoplasia, depressed nasal bridge, anteverted nares, bifid uvula, cleft hard palate, and micrognathia were slightly different among cases. The parents in all three families did not have any STL-associated phenotypes.

Conclusions: Our report substantially expands the molecular genetic basis of autosomal recessive STL and confirms the clinical phenotypes of *COL9A3*-associated autosomal recessive STL.Keywords: Stickler syndrome, *COL9A3*, Autosomal recessive

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P02.069.B Clinical and molecular revaluation yield a 52% of characterization in syndromic retinal diseases

Irene Perea-Romero[1,2], *Fiona Blanco-Kelly*[1,2], *Iker Sanchez-Navarro*[1], *Isabel Lorda-Sanchez*[1,2], *Saoud Tahsin-Swafiri*[1,2], *Almudena Avila-Fernandez*[1,2], *Inmaculada Martin-Merida*[1,2], *Maria Jose Trujillo-Tiebas*[1,2], *Rosario Lopez-Rodriguez*[1,2], *Ionut Florin Iancu*[1,2], *Raquel Romero*[1,2], *Mathieu Quinodoz*[3,4,5], *Pablo Minguez*[1,2], *Marta Corton*[1,2], *Carlo Rivolta*[3,4,5], *Carmen Ayuso*[1,2]

[1]*Health Research Institute-Fundación Jiménez Díaz University Hospital, Universidad Autónoma de Madrid (IIS-FJD, UAM), Madrid, Spain,* [2]*Center for Biomedical Network Research on Rare Diseases (CIBERER), Instituto de Salud Carlos III, Madrid, Spain,* [3]*Institute of Molecular and Clinical Ophthalmology Basel (IOB), Basel, Switzerland,* [4]*Department of Ophthalmology, University of Basel, Basel, Switzerland,* [5]*Department of Genetics and Genome Biology, University of Leicester, Leicester, United Kingdom.*

Introduction: Syndromic retinal diseases (SRD) are a group of rare and complex inherited systemic disorders, characterized by a challenging molecular study and clinical management.

Materials and Methods: A cohort study was performed on 100 index cases with an a priori diagnosis of non-Usher SRD, using available clinical and familiar data and HPO (Human Phenotype Ontology) annotation, so every case was classified into 7 different clinical categories according to the most prominent symptoms. Over the years, diverse molecular and bioinformatic methods have been used. Accordingly, cases were classified into 3 subgroups depending on the previous molecular technique utilized, determining the new approach to perform.

Results: After the phenotypic classification, the most common SRD were ciliopathies (36%). A characterization rate of 52% was obtained, being 6 cases incompletely characterized with a gene that partially explained the phenotype. An increased characterization rate was achieved taking the naïve (67.4%) or the ciliopathies (66.7%) subgroups independently. Our results suggest that customized panels and clinical exome would be the first-tier approach in the naïve cases, whereas whole-exome sequencing would be in the re-studied and reanalysed. Due to the causative gene found, 27.3% of the completely characterized cases were reclassified into another clinical subgroup.

Conclusions. Our study provides an example of SRD in the daily clinical practice and the importance of a thorough clinical analysis and election of the molecular approach, in order to solve these complex cases and elucidate new possible phenotype-genotype associations.

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P02.070.C Could TGFBI-related corneal dystrophies be mimicked in zebrafish via CRISPR/Cas9-mediated hot spot arginine variations?

Fulya Yaylacioglu Tuncay[1], *Beril Talim*[2], *Pervin Rukiye Dinçer*[3]

[1]*Department of Medical Biology, Gulhane Medical Faculty, University of Health Sciences, Ankara, Turkey,* [2]*Department of Pediatrics, Faculty of Medicine, Hacettepe University, Ankara, Turkey,* [3]*Department of Medical Biology,Faculty of Medicine, Hacettepe University, Ankara, Turkey.*

Introduction: TGFBI-related corneal dystrophies are characterized by hyaline or amyloid deposition in the cornea due to missense mutations of *TGFBI*. The protein product of this gene (TGFBIp) was shown in the cornea of zebrafish. Mutations of *TGFBI* affecting arginine residue on the 124th position were reported as one of the hot spots for this group of corneal dystrophies. This arginine residue was also conserved in zebrafish. Therefore, we aimed to make an in-frame change in zebrafish genome affecting this arginine residue by using CRISPR/Cas9 and then observe if any accumulation would occur in the cornea of adult zebrafish.

Materials and Methods: For the in-frame change in the target region nine injection groups were planned. The mixtures consisting of sgRNAs, Cas9 mRNA/protein with or without donor DNA were injected to one-cell stage embryos in each group ($n = 100$). T7 endonuclease assay, restriction fragment length polymorphism assay and Sanger sequencing were used for genotyping in the injection groups.

Results: The variation p. Ser115_Arg117delinsLeu (c. 347_353delinsT) was detected in one of the injection groups in F1 embryos. This variation deleted the target arginine in TGFBIp without causing frameshift. Three-months old and 1-year old heterozygote zebrafish were euthanized, and corneas were examined under the light microscope. No deposits could be detected with hematoxylin-eosin, Masson's trichrome and congo red staining.

Conclusion: This is the first study in literature that succeeded to make an in-frame variation effecting the hot spot arginine residue of TGFBIp in zebrafish. However, this success could not result in deposit formation in zebrafish cornea.

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P02.071.D An USH2A founder mutation is the cause of Usher syndrome type 2 in Sardinia

Vincenzo Rallo[1,2], *Rita Serra*[1,2], *Maristella Steri*[2], *Stefania Olla*[2], *Michele Marongiu*[2], *Edoardo Fiorillo*[2], *Antonio Pinna*[1], *Francesco Cucca*[1], *Andrea Angius*[1,2]

[1]*University of Sassari, Sassari, Italy,* [2]*Institute for Genetic and Biomedical Research (IRGB), Monserrato (Cagliari), Italy.*

Introduction: Usher syndrome (USH) is the most common cause of combined sight and hearing loss, responsible for half of deaf-blindness cases. USH has divided into three types: USH1, characterized by severe bilateral hearing loss and early retinitis pigmentosa (RP); USH2, distinguished by moderate hearing loss and RP; USH3, showing progressive hearing loss, vestibular dysfunction and RP. USH is associated with 19 loci, with 16 causative genes identified. Here, we describe the clinical findings and molecular analysis of 3 USH-affected unrelated families living in the island of Sardinia (Italy).

Materials and Methods: We investigated all the members of 3 families in which 9 patients showed both sensorineural hearing loss and RP. All individuals underwent a complete ophthalmic examination and vestibular medical tests. Whole-genome sequencing data were available for genetic analysis.

Results: Clinical hypothesis indicated a suspected USH2 syndrome. We identified a single missense causal variant in the USH2A gene, in homozygous status in all patients and heterozygous in unaffected parents. Mutation-related haplotype reconstruction revealed a founder effect. To understand if this event was restricted to a geographical area or whole island, we analysed about 3500 Sardinians, revealing a frequency of about 2% heterozygotes distributed overall in Sardinia.

Conclusions: By using our approach, we were able to describe the first case of USH syndrome, its incidence and distribution in Sardinia. Thus, we are able to provide an attractive perspective for the feasibility of carrier status screening, possible genetic counselling at the base for prevention and early treatment strategies of this hereditary syndrome.

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P03 Internal Organs & Endocrinology (Lung, Kidney, Liver, Gastrointestinal)

P03.001.A Molecular analysis of *PKD1* and *PKD2* genes: a key for achieving a great diagnosis rate in autosomal dominant polycystic kidney disease

Elena Mesa-Rísquez, *Alfonso Andújar, Isabel Sánchez-Guiu, Anna Baquero-Vaquer, Tania Otero-Rodríguez, María Dolores Ruiz, Roser Martínez-Rubio, Amparo Girós, María Sánchez-Ibáñez, Vanesa Felipe-Ponce, Dan Diego-Álvarez, Rosario Ferrer-Avargues, Uxía Esperón, Natalí Riva, Bárbara Masotto, Yolanda Moreno-Sáez, José Leonardo Díaz, Jaime García, Carmen Collado, María Dolores Oliver, Norma Aliaga, Angela Gaspar, Laura Cano, Ana Perpiñán, Nuria Serrano, Clara Casañ, Celia Buades-Gomis, Alejandro Romera-López, Mayte Gil-Borja, Sonia Santillán, Christian Martín Moya*

Ascires Sistemas Genómicos, Paterna (Valencia), Spain.

Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited kidney disease, mainly caused by *PKD1* and *PKD2* genes. *PKD1* gene analysis is technically complex due to its large size and the presence of several pseudogenes. The aim of this work is to present the diagnostic results of a ADPKD cohort.

143 ADPKD cases were included, of which 71.6% had family history of ADPKD. Patients were screened for *PKD1* and *PKD2* genes using Long Range PCR and Sanger sequencing or Nextera™ Technology for NGS. In some patients, a MLPA study was performed if a negative result was obtained from sequencing.

Genetic screening revealed 88 diagnosed patients and 55 undiagnosed, 27 of whom were carriers of Variants of Uncertain Significance (VUS) and the remaining 28 were negative. Disease-causing variants were either found in *PKD1* (65 cases) or *PKD2* (23 cases). Among the disease-causing variants in both genes, 25% of them were novel variants. Diagnostic variant effects were mainly nonsense (37.5%) and small deletions (26.14%). Other types of identified variants included small insertions (12.5%), missense (11.36%), splicing (7.95%), gross deletions (3.41%) and small indels (1.14%).

Our rate of 61.54% positive genetic diagnosis highlights the effectiveness of molecular genetic analysis as a powerful tool for achieving a diagnosis in ADPKD patients. NGS allows *PKD1* and *PKD2* simultaneous analysis, reducing processing time and costs. Extending the genetic analysis by exome directed panels to related genes as *GANAB*, *DNAJB11* or performing cosegregation and functional studies of VUS could lead to a higher diagnostic rate.

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P03.003.C The mild effect of the *COL4A5* variant p.Gly624Asp in a group of 15 Polish patients with Alport syndrome

Paulina Halat-Wolska[1], *Elżbieta Ciara*[1], *Lukasz Obrycki*[2], *Katarzyna Gadomska-Prokop*[2], *Dorota Piekutowska-Abramczuk*[1], *Joanna Kosińska*[3], *Małgorzata Rydzanicz*[3], *Piotr Stawiński*[3,4], *Beata Chałupczyńska*[1], *Kamila Frączak*[1], *Marzena Gawlik*[1], *Dorota Jurkiewicz*[1], *Paweł Kowalski*[1], *Magdalena Pelc*[1], *Dorota Siestrzykowska*[1], *Szymon Szyszkowski*[1], *Dorota Wicher*[1], *Ryszard Grenda*[2], *Krystyna Chrzanowska*[1], *Rafał Płoski*[3], *Mieczysław Litwin*[2]

[1]*Department of Medical Genetics, The Children's Memorial Health Institute, Warsaw, Poland,* [2]*Department of Nephrology, The Children's Memorial Health Institute, Warsaw, Poland,* [3]*Department of Medical Genetics, Warsaw Medical University, Warsaw, Poland,* [4]*Department of Genetics, Institute of Physiology and Pathology of Hearing, Warsaw, Poland.*

Introduction: Alport syndrome (AS) is a clinically and genetically heterogeneous nephropathy caused by pathogenic variants in *COL4A3, COL4A4* or *COL4A5*. About 85% of AS patients have X-linked inheritance, which causes more severe phenotype in males, whereas in females, penetrance depends on X-chromosome inactivation pattern. Originating in the Middle Ages *COL4A5* variant c.1871G>A p.Gly624Asp is predominant in Central/East Europe and mostly gives mild AS symptoms.

Materials and Methods: Next-generation sequencing analysis of glomerulopathy and chronic kidney disease related genes panel was performed in Polish patients with suspected AS.

Results: In 65 patients clinical diagnosis was confirmed at the molecular level. The most frequent was X-linked AS caused by 32 different *COL4A5* variants (75%). A recurrent *COL4A5* variant p.Gly624Asp in 15 patients (nine males and six females) was identified. Additionally, four patients with this substitution had pathogenic variant in *COL4A3*, *HNF1B* or *MYH9*. The clinical course of patients with this genotype was mostly milder than observed in individuals with other *COL4A5* variants. They presented only haematuria with or without proteinuria. Of these, two patients also had hearing impairment: male with only p.Gly624Asp variant and female with additional variant in *COL4A3*.

Conclusions: The results of this study broaden the genotypic spectrum of AS, which will facilitate future research on the genotype-phenotype correlations. Variant p.Gly624Asp in *COL4A5* was predominant and accounted for 31% of X-linked AS in the study group. Observations of mild phenotype in our patients with this genotype relate to literature data. **Partially supported: CMHI-M29/18**

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P03.004.A Delineation of the phenotypic and genotypic spectrum of type-IV-collagen-related nephropathy - Alport syndrome and thin basement membrane nephropathy

Korbinian M. Riedhammer[1,2], *Matthias C. Braunisch*[2], *Jasmina Ćomić*[1], *Adrian Lungu*[3], *Jovana Putnik*[4], *Gordana Miloševski-Lomić*[5],

Michaela Gessner[6], Nataša Stajić[4], Ludwig Patzer[7], Nora Emini[8], Velibor Tasic[8], Julia Hoefele[1]

[1]Institute of Human Genetics, Klinikum rechts der Isar, School of Medicine, Technical University of Munich, Munich, Germany, [2]Department of Nephrology, Klinikum rechts der Isar, School of Medicine, Technical University of Munich, Munich, Germany, [3]Pediatric Nephrology Department, Fundeni Clinical Institute, Bucharest, Romania, [4]Institute for Mother and Child Health Care of Serbia "Dr Vukan Čupić", Department of Nephrology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia, [5]Department of Nephrology, University Children's Hospital, Belgrade, Serbia, [6]Department of General Pediatrics and Hematology/Oncology, Children's University Hospital Tuebingen, Tuebingen, Germany, [7]Children's Hospital St. Elisabeth and St. Barbara, Halle (Saale), Germany, [8]University Children's Hospital, Medical School Skopje, Skopje, Macedonia, The Former Yugoslav Republic of.

Introduction: "Type-IV-collagen-related nephropathy" describes a spectrum of hereditary hematuric diseases comprising Alport syndrome (AS) and (milder) thin basement membrane nephropathy (TBMN). AS results from biallelic causative variants in *COL4A3/4* (autosomal recessive AS) and hemizygous causative variants in *COL4A5* (X-linked AS). Females with heterozygous causative variants in *COL4A5* show a variable phenotype. Monoallelic causative variants in *COL4A3/4* are identified in TBMN. The designation "autosomal dominant AS" for heterozygous carriers of causative variants in *COL4A3/4* is contested.

Methods: 64 index patients, in whom exome sequencing had been performed, were assigned to a TBMN (18 cases) or AS (46 cases) subgroup based on clinical/histopathologic presentation and family history. Phenotypic and genotypic features were compared.

Results: Diagnostic yield of type-IV-collagen-related nephropathy classified as AS compared to TBMN was significantly different (65% vs. 28%; $p = 0.01$). One case, clinically classified as TBMN, had Dent disease genetically. The further solved TBMN cases carried heterozygous causative variants in *COL4A3*, *COL4A4* or *COL4A5* (female). Median age at first manifestation was significantly lower in AS compared to TBMN cases (5.5 years vs. 16.0 years; $p = 0.001$). TBMN cases had no extrarenal manifestations, in contrast to 28% of AS cases ($p = 0.01$). 39% of TBMN cases had a reported family history in contrast to 78% in AS cases ($p = 0.006$).

Conclusions: This study delineates the phenotypic and genotypic spectrum of type-IV-collagen-related nephropathy. Importantly, it takes the perspective of the clinician who has to integrate phenotypic and anamnestic data to assess the possibility of a hereditary disease.

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P03.005.A Systematic variant reinterpretation in patients with type-IV-collagen-related nephropathy (Alport syndrome/thin basement membrane nephropathy) reveals a high rate of ambiguous results

*Korbinian M. Riedhammer[1,2], **Patrick Richthammer**[1], Dominik S. Westphal[1], Jasmina Ćomić[1], Roman Günthner[2], Matthias C. Braunisch[2], Sabine Rath[3], Anja K. Büscher[4], Hanns-Georg Klein[3], Stefanie Weber[5], Julia Hoefele[1]*

[1]Institute of Human Genetics, Klinikum rechts der Isar, Technical University of Munich, School of Medicine, Munich, Germany, [2]Department of Nephrology, Klinikum rechts der Isar, Technical University of Munich, School of Medicine, Munich, Germany, [3]Center for Human Genetics and Laboratory Diagnostics Dr. Klein, Dr. Rost and Colleagues, Martinsried, Germany, [4]Pediatric Nephrology, Pediatrics II, University Children's Hospital Essen, Essen, Germany, [5]University Children's Hospital, Marburg, Germany.

Introduction: Type-IV-collagen-related nephropathy covers a spectrum of hereditary hematuric diseases (thin basement membrane nephropathy, TBMN; Alport syndrome, AS). Whereas AS results from disease-causing variants in *COL4A3-5* (autosomal recessive and X-linked AS), monoallelic disease-causing variants in *COL4A3/4* are associated with TBMN.

Methods: Variants of 96 index cases reported between 2009 and 2014 with the clinical tentative diagnosis AS (77/96), TBMN (13/96) or unclear determination of AS/TBMN (6/96) were meticulously reinterpreted based on ACMG criteria and current amendments. Monoallelic (likely) pathogenic variants in *COL4A3/4* in an AS case were not considered as diagnostic, as the designation "autosomal dominant AS" is contested. 6 cases had to be excluded from further analysis due to limited data.

Results: In total, 84 variants (allocated to 96 cases) and their genotypes have been reassessed (*COL4A3*: 21/84, *COL4A4*: 18/84, *COL4A5*: 45/84). 64/90 cases included in the further analysis could be classified as solved (original report: 83/90; $p < 0.001$, Fisher exact test). 26/90 were classified as "not solved" (original report: 7/90), due to inconclusive results on variant (11/90), on genotype (12/90) or both variant and genotype level (3/90).

Conclusions: Transparent and coherent variant-/genotype-interpretation enables physicians to diagnose hereditary diseases and allows them to provide patients with well-founded information concerning prognosis and recurrence risk. This is especially true for type-IV-collagen-related nephropathy, covering an intricate phenotypic and genotypic spectrum. Since reassessment of variants/genotypes in this study showed a significant reduction in genetic diagnoses, reports on type-IV-collagen-related nephropathy obtained in the pre-ACMG era should be critically evaluated.

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P03.006.B Impact of human genetic variants on C-Reactive Protein levels and acute appendicitis

Isis Ricaño-Ponce[1], *Toon Peeters[1,2,3], Vasiliki Matzaraki[1], Mihai Netea[1,4], Inge Gyssens[1,2,3], Vinod Kumar[1,5]*

[1]Department of Internal Medicine and Radboud Center for Infectious Diseases, Radboud University Medical Center, NIJMEGEN, Netherlands, [2]Department of Infectious Diseases & Immunity, Jessa Hospital, Hasselt, Belgium, [3]Faculty of Medicine and Life Sciences, Hasselt University, Hasselt, Belgium, [4]Human Genomics Laboratory, Craiova University of Medicine and Pharmacy, Craiova, Romania, [5]University of Groningen, University Medical Center Groningen, Department of Genetics, Groningen, Netherlands.

Introduction: Acute appendicitis is one of the most common abdominal emergencies worldwide. Both environmental and genetic factors contribute to disease. C-Reactive protein is (CRP) is one of the most important biomarkers in the diagnosis of acute appendicitis. CRP-levels are significantly affected by genetic variation. However, it remains unclear whether such genetic variation is causally related to appendicitis risk. Therefore, in this study, we investigate the causal relationship between SNPs

associated with circulating CRP-levels and the risk and severity of acute appendicitis.

Materials and Methods: we measured CRP-levels in serum of appendectomy patients (n = 325) with appendicitis severity categorised as complicated/uncomplicated. We also performed GWAS in the same group of patients. We performed intersection, colocalization of our GWAS results with appendicitis and CRP-associated loci from the Pan-UKBB cohort. We employed pathway enrichment analyses and functional-genomics approach to prioritise genes and pathways.

Results: We observed a significant enrichment of CRP-loci in SNPs associated to appendicitis and complicated appendicitis. Among these shared loci, we characterise two top loci at 1q41 and at 8p23.1. The *HLX* gene at 1q41 is involved in blood cells differentiation, liver and gut organogenesis. The locus at 8p23.1 affects multiple genes that are overexpressed in appendix tissue from appendicitis patients. Using a functional genomics approach we provide genetic evidence for the involvement of interferon signalling pathway in complicated appendicitis.

Conclusions: Our results suggest shared genetic mechanisms between appendicitis and CRP-levels. By identifying new pathways, our study identified genetic causes for severity of appendicitis.

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P03.008.D Novel *CTLA4* heterozygous mutation in a family with type 2 autoimmune polyendocrine syndrome

Federico Romani[1], *Emanuele Micaglio*[1], *Filippo Martinelli Boneschi*[2], *Giorgio Nevio Casari*[3], *Sara Benedetti*[3], *Paola Carrera*[3], *Carlo Pappone*[1]

[1]*IRCCS Policlinico San Donato, San Donato Milanese, Italy,* [2]*IRCCS Ospedale Maggiore Policlinico, Milan, Italy,* [3]*IRCCS San Raffaele Hospital, Milan, Italy.*

Autoimmune polyendocrine syndromes are an emerging field in current medicine due to both diagnostic and therapeutic improvements. Although generally accepted as complex traits, many genes play a pivotal role in both pathogenesis and prognosis of autoimmune polyendocrine syndromes. Among those genes, *CTLA4* has been described as very important for autoimmunity against endocrine tissues since 2004. We describe a family in which a heterozygous variant in *CTLA4* gene segregates with a type 2 autoimmune polyendocrine overt phenotype. Both the proband and his sister are indeed affected by recurrent oral candidiasis, hypothyroidism, type 1 diabetes mellitus and Addison disease. Interestingly, in both patients the onset of clinical picture happened with a recurrent oral candidiasis, in absence of genetic mutation in MODY genes. Proband and sister carry a c.475G>C heterozygous mutation of *CTLA4* gene, with unknown inheritance. The bioinformatic analysis suggested a pathogenic effect likely related to the absence of the described mutation in healthy people. This conclusion is supported by the segregation with the pathologic phenotype in the examined family. To the best of our knowledge, this is the first case of *CTLA4* heterozygous mutation in a clinically confirmed family affected by type 2 autoimmune polyendocrine syndrome. Although proband's parents were not available for the genetic analysis, this case highlights the growing relevance of genetic testing for this condition. Moreover, our data suggest a possible new genotype - phenotype correlation requiring further studies in order to increase the diagnostic yield of genetic testing in autoimmune polyendocrine syndromes.

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P03.009.A Association of KEAP1 polymorphism with autoimmune thyroiditis

Cristina Robles Lázaro[1,2], ***María Ovejero-Sánchez***[3,2,4], *Nerea Gestoso-Uzal*[2,3,4], *Carlos Gutiérrez-Cerrajero*[2,3,4], *Paloma Martín-Bejarano Soto*[2,3,4], *Pamela Vázquez-Cárdenas*[3,2,4], *Ana Belén Herrero*[2,3,4], *Rogelio González-Sarmiento*[2,3,4]

[1]*Endocrinology Service, University Hospital of Salamanca, Salamanca, Spain,* [2]*Molecular Medicine Unit, Department of Medicine, University of Salamanca, Salamanca, Spain,* [3]*Institute of Biomedical Research (IBSAL), Salamanca, Spain,* [4]*Institute of Molecular and Cellular Biology of Cancer (IBMCC), University of Salamanca-CSIC, Salamanca, Spain.*

Introduction: Autoimmune thyroiditis is a chronic inflammatory process characterized by the presence of glandular lymphocytic infiltration and thyroid specific antibodies. Some studies suggest that oxidative stress could be involved in the development of this disease. Keap1-Nrf2 pathway is the major regulator of cytoprotective responses to oxidative and electrophilic stress. Thus, the main aim of this study is to determine if Single Nucleotide Polymorphisms (SNPs) in these genes could be associated with a higher risk of developing autoimmune thyroiditis.

Material and methods: 202 autoimmune thyroiditis patients and 340 healthy subjects without previous history of autoimmune disease were recruited. Genotyping of SNPs in Keap1 (rs1048290 and rs11545829) and NRF2 (rs2706110) was performed using TaqMan allelic discrimination assays. Odd ratios and 95% confidence intervals were estimated for each polymorphic variant to evaluate the association with risk of developing autoimmune thyroiditis, with p-values < 0.05 being considered statistically significant.

Results: The analysis of genotypic and allelic distribution of rs1048290 SNP in Keap1 gene and rs2706110 SNP in NRF2 gene did not show statistically significant differences between both groups of subjects. However, the heterozygous GA genotype of the Keap1 rs11545829 polymorphism was associated with increased risk of developing autoimmune thyroiditis. Also, the statistical analysis showed that G allele confers protection from this disease.

Conclusion: Our study suggests that the genotype AG in the polymorphism rs11545829 of Keap1 gene could increase the risk of developing autoimmune thyroiditis due to an alteration of the cellular response to oxidative stress.

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P03.010.B The use of high throughput sequencing for the identification of variants contributing to autosomal dominant polycystic kidney disease in the Maltese population

Maria Cini Masini[1], *Francesca Borg Carbott*[1], *Ritienne Attard*[1], *Adrian Pleven*[1], *Karen Cassar*[2], *Roberta A. Callus*[3], *Angela Borg Cauchi*[3], *Valerie Said Conti*[4], *Stephanie Bezzina Wettinger*[1], *Rosienne Farrugia*[1]

[1]Department of Applied Biomedical Science, Faculty of Health Sciences, University of Malta, L-Imsida, Malta, [2]Department of Medicine, Faculty of Medicine and Surgery, University of Malta, L-Imsida, Malta, [3]Renal Division, Department of Medicine, Mater Dei Hospital, L-Imsida, Malta, [4]Department of Paediatrics, Mater Dei Hospital, L-Imsida, Malta.

Introduction: Autosomal dominant polycystic Kidney Disease (ADPKD) though rare, is the most common hereditary kidney disease. It is characterized by enlarged kidneys, bilateral formation and progressive expansion of renal cysts, as well as systemic manifestations from the progression of renal disease requiring renal replacement therapy or transplantation.

Materials and Methods: DNA samples were obtained from 16 recruited probands and 6 additional affected family members as part of the Malta NGS Project. We used Agilent SureSelect^{XT} Targeted Enrichment gene panel and high throughput sequencing (HTS) on Illumina HiSeq4000 for 3 nuclear families. Whole exome sequencing was carried out on another 15 probands. HTS data was mapped to GRCh37 as paired-end libraries using NextGENe® software. After variant filtering and prioritization, variants were confirmed by Sanger sequencing using primers unique to *PKD1* or *PKD2*.

Results: We identified 13 variants in *PKD1;* two frameshifts, seven missense changes (2 novel), and four nonsense mutations (2 novel). Each *PKD1* variant was identified in a single family, and one family had 2 linked variants. A *PKD2* splice site mutation, was identified in two unrelated probands. For two patients no causative variant was identified in known ADPKD candidate genes.

Conclusion: This study demonstrates that laborious long-range PCRs of *PKD1* and *PKD2* may be replaced by HTS and stringent data analysis reducing cost and analysis time.

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P03.013.A Association between cortisol deficiency and life threatening arrhythmia

Afaf Alsubhi[1], *Mohammed Aldubayee*[2], *Wafaa Eyaid*[1]

[1]Department of Pediatrics, Genetics division, King AbdulAziz Medical City, RIYADH, Saudi Arabia, [2]Department of Pediatrics, King Abdullah Specialized Children's Hospital (KASCH), RIYADH, Saudi Arabia.

A 5 days old full term baby girl was admitted to our pediatric intensive care unit with non ketotic hypoglycemia, abnormal movement along with bradycardia and progressive prolongation of the QT interval. The Whole exome sequencing showed likely pathogenic variant in the TBX19 gene that is associated with congenital isolated adrenocorticotropic hormone deficiency that was proven biochemically. She was treated with hydrocortisone and showed dramatic improvement of her QT interval length. After discharge, she lost follow up. She was readmitted again at the age of 16 months with similar symptoms that improved after restarting hydrocortisone therapy with improvement of the EKG findings and bradycardia. In the literature there is one case report of an adult patient with similar association between central cortisol deficiency and life-threatening arrhythmia.Hypoglycemia per se does not cause a prolonged QT interval; this indicates that cortisol deficiency might be the cause of this patient presentation.

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P03.014.B A novel homozygous missense variant in *PTPN2* associated with early onset Crohn's disease and growth failure

Tony Yammine, *Alain Chebly, Nabiha Salem, Rita Esber, Mirna Souaid, Chantal Farra*

Medical Genetics Unit, Beirut, Lebanon.

Crohn's disease (CD) is a chronic idiopathic inflammatory bowel disease that can affect any part of the gastrointestinal tract. Major manifestations are diarrhea, abdominal pain, weight loss with or without fever. CD is a multifactorial disorder where genetic factors are important players. Several candidate loci or genes including PTPN2 (#MIM 176887) have been reportedly associated with CD. PTPN2 is a major contributor in controlling inflammatory signaling cascades and maintaining intestinal barrier functions. We conducted whole-exome sequencing in a 9-year-old Lebanese girl with a CD onset at 13 months and in both her asymptomatic parents. Analysis detected a novel homozygous variant in PTPN2: c.359C>T, p.(Ser120Leu) in the patient, while both her parents were heterozygous for the variant. c.359C>T is located in the protein tyrosine phosphatase domain within a highly conserved amino acid. It is classified as likely pathogenic according to the ACMG criteria, deleterious by SIFT and disease causing by Mutation Taster. In order to evaluate the hypothetical functional consequences of the identified variant, we performed a quantitative expression analysis of PTPN2 in blood tissues from the patient, her parents and two healthy controls. An almost absent PTPN2 expression was noted in the patient compared to her parents and the controls, suggesting a functional PTPN2 impairment caused by c.359C>T. This is the first time, a homozygous PTPN2 variant is associated with an early onset CD. Further reports with PTPN2 gene variants would enable a better delineation of the molecular basis of CD.

T. Yammine: A. Employment (full or part-time); Significant; Saint Joseph University. **A. Chebly:** A. Employment (full or part-time); Significant; Saint Joseph University. **N. Salem:** A. Employment (full or part-time); Significant; Saint Joseph University. **R. Esber:** A. Employment (full or part-time); Significant; Saint Joseph University. **M. Souaid:** A. Employment (full or part-time); Significant; Saint Joseph University. **C. Farra:** A. Employment (full or part-time); Significant; Saint Joseph University, Hotel Dieu de France.

P03.015.C Spectrum of *CFTR* variants in patients with cystic fibrosis revealed in Western Siberian Ugra region (Russia)

Maxim Donnikov[1,2], *Vitaly Mescheryakov*[1], *Dmitry Lozhkin*[2], *Lev Kolbasin*[2], *Andrey Glotov*[3], *Oleg Glotov*[4], *Irina Urvantseva*[5], *Lyudmila Kovalenko*[1]

[1]Medical Institute of Surgut State University, Surgut, Russian Federation, [2]Medical Genetics Counseling Service of Diagnostics and Cardiovascular Surgery Center (Cardiology Clinic) of KHMAO-Ugra, Surgut, Russian Federation, [3]Department of Genomic Medicine, D. O. Ott Research Institute of Obstetrics, Gynecology and Reproduction, Saint-Petersburg, Russian Federation, [4]City Hospital 40, Saint-Petersburg, Russian Federation, [5]Diagnostics and

Cardiovascular Surgery Center (Cardiology Clinic) of KHMAO-Ugra, Surgut, Russian Federation.

Introduction: During the era of cystic fibrosis (CF) target therapy knowing population structure of *CFTR* variants is of immense need. Applying battery of molecular genetics tests in routine practice of regional genetics laboratory allowed to perform extensive analysis of *CFTR* variants in regional CF patients.

Materials and methods: gDNA obtained from 57 CF patients (1998-2020 yob) from regional CF registry by column extraction; HRMA performed using Bio-Rad software; MLPA and Sanger sequencing performed on GenomeLab GeXP (Beckman-Coulter); NGS performed on MiSeq using NimbleGen SeqCap_EZ_CysFib kit.

Results: Total of 117 *CFTR* alleles were revealed (see Table), among them 6 major regional mutations covering 69.2% of all alleles, with most frequent [delta]F508 variant. 28 variants of different types (mostly missense and nonsense) with frequency of 0.9% each (found only in 1 patient each) comprise 23.9% of all alleles. If classified by type of nucleotide sequence change, the spectrum contains 50.4% of ins/del without frameshift mutations ([delta]F508 as representative); 20.5% of missense variants (E92K); 9.4% nonsense (S466X); 9.4% ins/del with frameshift (1677delTA); 5.1% large rearrangements (dele2,3); 3.4% VUCS; 1.7% splicing defects.

Conclusions: If ranged, *CFTR* variants spectrum from regional CF cases resembles that of common Russian alleles for 5 major variants (except R1066C, listed in Table), while sharing only 2 major mutations with Europe ([delta]F508, N1303K). High prevalence of rare variants dictates the necessity of sequencing.

List of CFTR variants in CF patients from Ugra

	CFTR variant calling (HGVS; legacy)	type of variant	n = 117	%
1	c.1521_1523delCTT ([delta]F508)	del w/o frameshift	57	48.7
2	c.54-5940_273+10250del21kb (CFTRdele2,3)	large rearrangement	6	5.1
3	c.1545_1546delTA (1677delTA)	del w/ frameshift	6	5.1
4	c.274G>A (E92K)	missense; splicing defect	5	4.3
5	c.3196C>T (R1066C)	missense	4	3.4
6	c.3909C>G (N1303K)	missense	3	2.6
7	c.412_413insACT (L138ins)	ins w/o frameshift	2	1.7
8	c.1397C>G (S466X)*	nonsense	2	1.7
9	c.1399C>T (R1070Q)*	missense	2	1.7
10	c.3209G>A (L467F)	VUCS	2	1.7
11-38	28 variants (each 0,9%)	*all*	28	23.9

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P03.017.A Frequnecy of CFTR mutations in a Romanian cohort of individuals with cystic fibrosis

Georgiana Ilie[1], *Viorica Radoi*[2,3], *Andreea Ionescu*[4], *Iuliana Chelu*[4], *Cristina Dragomir*[4], *Gratiela Chelu*[4], *Mihaela Popa*[4], *Bianca Basangiu*[4], *Andra Perioc*[4], *Oana Istrate*[4], *Traian Grozescu*[2], *Paul Iordache*[5], *Lucian Pop*[3], *Natalia Cucu*[6], *Camil Laurentiu Bohiltea*[2,3], *Radu-Ioan Ursu*[2,3,4]

[1]"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania, [2]"Carol Davila" University of Medicine and Pharmacy, Department of Medical Genetics, Bucharest, Romania, [3]National Institute for Mother and Child Health "Alessandrescu-Rusescu", Bucharest, Romania, [4]Synevo Romania, Department of Medical Genetics, Chiajna, Romania, [5]"Carol Davila" University of Medicine and Pharmacy, Department of Epidemiology, Bucharest, Romania, [6]University of Bucharest, Faculty of Biology, Department of Genetics, Bucharest, Romania.*

Background: Cystic fibrosis is one the most common autosomal-recessive genetic disorders in the Caucasian population, caused by homozygous or compound heterozygous mutations in the CFTR gene (on the long arm of chromosome 7).

Material. Methods: The study group includes 74 patients with the clinical diagnosis of cystic fibrosis and 132 healthy relatives of patients with cystic fibrosis (carrier screening testing). The testing method was PCR using a panel including the most common 38 mutations in CFTR gene in the European population and the 5T-7T allele polymorphism.

Results: All the patients with the clinical diagnosis of cystic fibrosis were positive for mutations in the CFTR gene, in a homozygous or in a heterozigous compound state. Of all these pacients, 56.75% (42/74) had the frameshift deltaF508 mutation, followed by other common mutations in the studied group, such as the G551D, the R553X or the R1158X variants. 57 out of the total of 132 suspected healthy carriers (43.2%) were positive for CFTR mutations in a heterozygous status. The most prevalent mutation was in this case also the deltaF508 genetic variant. Rarer mutations, such as the W1282X and the c.711+1G>A variants, were detected in 1 patient each.

Conclusions: The results of the study are concordant with the reports in literature regarding the prevalence of CFTR mutations in the Eastern-European population. Genetic testing for CFTR mutations is important not only for the genetic diagnosis of the disease, but also for the identification of heterozygous carriers or individuals at risk of passing the disease on.

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P03.018.B Late diagnosisof cystic fibrosisin a 59-year-old patient

Elena Zhekaite[1], *Elena Amelina*[2], *Elena Kondratyeva*[1], *Tagui Adyan*[1]

[1]Research Centre for Medical Genetics, Moscow, Russian Federation, [2]Scientific Research Institute of Pulmonology of the Federal Medical and Biological Agency of Russia, Moscow, Russian Federation.*

Introduction: According to Russian CF Patient Registry 2018 the average age of CF patients in Russia was 12.8 ± 9.6 years, the average age of diagnosis - 3.1 ± 6.1 years (4.1month in Europe according to European Cystic Fibrosis Society Patient Registry 2016). Few patients in Russia are over 55 years of age.

Materials and methods: The patient had atypical clinical symptoms since childhood (chronic sinusitis mostly, polypotomy at the age of 17). For the last 15 years there were complaints of persistent cough, exacerbations of chronic bronchitis about 2 times a year. 3 years ago bronchiectasis were detected. Patient has no children, his profession - a worker at a steel plant. Physical examination revealed dyspnea, low BMI (19.2 kg/m2), severe bronchial obstruction (FVC 54%, FEV1 33%), bilateral bronchiectasis on CT.

Results: Sweat test (Nanoduct) was performed - conductivity 83mmol/l. DNA diagnostics in the gene CFTR identified 2 pathogenic variants - [c.1521_1523delCTT]; [c.413_415dupTAC].

Conclusions: Our clinical case shows that the patients with "mild" CFTR genotype can be diagnosed at any age, patients with bronchiectasis, chronic polysinusitis should be tested for CF.

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P03.019.C Familial case of Cystic Fibrosis with genotype-phenotype correlations

*Tinatin Tkemaladze[1,2,3], **Mariam Ghughunishvili**[4,5], Eka Kvaratskhelia[1,5], Elene Abzianidze[1], Volha Skrahina[3], Arndt Rolfs[3,6,7]*

[1]Department of Molecular and Medical Genetics, Tbilisi State Medical University, Tbilisi, Georgia, [2]G. Zhvania Pediatric Academic Clinic, Tbilisi State Medical University, Tbilisi, Georgia, [3]Centogene GmbH, Rostock, Germany, [4]G.Zhvania Pediatric Academic Clinic, Tbilisi State Medical University, Tbilisi, Georgia, [5]Bakhutashvili Institute of Medical Biotechnology, Tbilisi State Medical University, Tbilisi, Georgia, [6]University Rostock, Medical Faculty, Rostock, Germany, [7]Arcensus GmbH, Rostock, Germany.

Introduction: Cystic Fibrosis (CF) is the most common recessive condition in Caucasians. Clinical expression of the disease is heterogeneous and no specific genotype-phenotype correlation had been observed. Certain *CFTR* variants are defined as "CF" alleles causing CF phenotype, and other alleles are defined as "risk factor" alleles, causing CBAVD, pancreatitis and atypical CF.

Case report: Here we describe a familial case of CF with three children: 11 yo and 8 yo siblings with classical CF phenotype had positive NBS and elevated sweat chloride test results. In younger 6 yo sibling NBS and sweat chloride test results were normal and she only had two episodes of airway infections and small weight.

Results: *CFTR* sequencing was performed for all three siblings: two older siblings with classical CF phenotype had 1677delTA and I1234V variants (both classified as "CF" alleles), whereas younger sibling had 1677delTA and L997F variants (classified as "CF" and "risk factor" alleles respectively). When the healthy parents' samples were analyzed the father turned out to have one heterozygous 1677delTA allele, and the mother had two variants - L997F and I1234V (classified as "risk factor" and "CF" alleles respectively). Currently mother is 40 yo and she is completely healthy.

Conclusion: To date none of the above-mentioned combination of alleles detected in the siblings and the mother are described in the cftr2.org database. We propose that combination of "CF" allele and "risk factor" allele does not cause CF and proper classification of variants is crucial for correct interpretation and diagnosis of the condition.

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P03.020.D Unusual presentation of CF in an infant

***Dario Portillo-Miño**, Efren Esteban Cerón-Muñoz*

Hospital Infantil Los Angeles, Pasto, Colombia.

Introduction: Cystic Fibrosis (CF) is the most common autosomal recessive, genetic condition in Caucasians, occurring in 1 of every 2,000 to 3,000 live births. Hepatic abnormalities in patients with CF have usually been reported in about 30-40% of the children. A multisystemic, progressive, and lethal disease is distinguished by the relationship of several organic components, mainly; pulmonary, pancreatic, and gastrointestinal.

Materials and methods: It is was the research in the clinic history, laboratory test, and molecular studies for the case. Besides, the search of the literature in PubMed, Google Scholar, Science Direct, LILACS, and Scielo. The case report is based on the CARE 2016 guideline.

Results: This case report attempts an approach to the clinical findings of hepatobiliary manifestations in CF. An infant was less than 1-month-old with an insidious clinical picture that debut with cholestasis and jaundice. The CFTR gene analysis results with mutation c.2353C>T (transition of cytosine by thiamin at position 2353 of cDNA), p.Arg785* (in the protein produces a change of arginine by stop codon), in a homozygous state, this change has been reported in the literature as a pathogenic change related to CF.

Discussion/Conclusion: CF is associated with liver involvement around 30%. In children, hepatobiliary symptoms occur at puberty when damage to the liver system is in advanced stages. The atypical presentation of CF with liver involvement is very rare and lethal in an infant. Understanding the different form of CF, it is essential for early diagnosis and to achieve integral management.

D. Portillo-Miño: None. **E. Cerón-Muñoz:** None.

P03.022.B Exome sequencing implicates heterozygous variant in *DSTYK* in functional urinary bladder disturbance

***Clara Vidic**[1], Marcin Zaniew[2], Holger Thiele[3], Janine Altmüller[3], Heiko Reutter[1,4], Alina C. Hilger[1,5]*

[1]Institute of Human Genetics, University of Bonn, Bonn, Germany, [2]Department of Pediatrics, University of Zielona Góra, Zielona Góra, Poland, [3]Cologne Center for Genomics, University of Cologne, Cologne, Germany, [4]Department of Neonatology and Pediatric Intensive Care, Children's Hospital, University of Bonn, Bonn, Germany, [5]Department of Pediatrics, Children's Hospital, University of Bonn, Bonn, Germany.

Introduction: *DSTYK* encodes dual serine/threonine and tyrosine protein kinase. *DSTYK* has been associated with autosomal dominant congenital anomalies of the kidney and urinary tract and with autosomal-recessive hereditary spastic paraplegia. Here we report a father and his two dizygotic twin sons with a novel, heterozygous variant in *DSTYK*, all presenting with voiding dysfunction due to functional urinary bladder disturbance.

Materials and Methods: We performed whole exome sequencing of the family. All three presenting clinically with hesitancy, abnormal voiding pattern and night incontinence till adolescence. In the sons cystoscopy excluded urethral valves but showed hypertrophy of the bladder neck and trabeculated bladder. Additionally, both sons were diagnosed with epilepsy. Based on the pedigree, filtering of exome data focused on rare (MAF < 0.01%), autosomal-dominant variants, predicted to be deleterious, residing in highly conserved regions of the exome. Validation of prioritized variants was performed using Sanger sequencing.

Results: We identified a novel, heterozygous c.271C>A (p.Leu91Met) variant in *DSTYK* segregating with the disease. The amino acid is highly conserved. Rated deleterious by 3 different prediction programs (SIFT, PolyPhen, MutationTaster) and with a CADD score of 24.5, this variant was prioritized as likely disease causing.

Conclusion: To the best of our knowledge, we describe the first familial case with autosomal dominant inherited variants in *DSTYK* and specific functional bladder outlet obstruction and epilepsy. Acknowledgements: C.V. is supported by BONFOR grant O-149.0133. A.C.H. is supported by BONFOR grant O-149.0123.

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P03.024.D Polymorphisms of glutathione synthetase gene are associated with susceptibility to type 2 diabetes and hyperglycemia

Iuliia Azarova, *Elena Klyosova, Ekaterina Shkurat, Alexey Polonikov*

Kursk State Medical University, Kursk, Russian Federation.

Background: The present study investigated whether single nucleotide polymorphisms (SNPs) in glutathione synthetase (*GSS*) gene, antioxidant enzyme involved in glutathione metabolism, contribute to type 2 diabetes (T2D) susceptibility.

Methods: A total of 3198 unrelated Russian subjects including 1572 T2D patients and 1626 sex- and age matched healthy subjects were enrolled for the study. Three common tagSNPs such as rs13041792, rs1801310 and rs6088660 of *GSS* were genotyped using the MassArray System. Plasma glutathione and reactive oxygen species (ROS) levels of study participants were measured by fluorometric and colorimetric assays, respectively.

Results: We found that SNP rs13041792 at the *GSS* gene is associated with an increased risk of T2D (OR 1.24, 95%CI 1.06-1.44, $P = 0.027$). A *GSS* haplotype rs1801310G-rs6088660C-rs13041792A (OR 1.26, 95CI 1.08-1.47, $P_{global} = 0.039$) showed a significant association with T2D risk. Genotype rs13041792-A/A was associated with increased levels of fasting blood glucose (FBG) in entire group of T2D patients ($P = 0.032$) and diabetic males ($P = 0.026$). Meanwhile, genotype rs1801310-A/A was associated with decreased levels of total glutathione in plasma in diabetic females ($P = 0.039$). Genotype rs6088660-T/T showed an association with decreased levels of FBG in males ($P = 0.007$) and decreased levels of ROS in females ($P = 0.039$).

Conclusion: We found for the first time that genetic polymorphisms at *GSS* gene are associated with susceptibility to type 2 diabetes and related hyperglycemia through the mechanisms involving decreased antioxidant defense and increased production of reactive oxygen species. The study was supported by Russian Science Foundation (№20-15-00227).

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P03.025.A Clinical utility of genetic testing in early-onset kidney disease: seven genes are the main players

Andrea Domingo Gallego[1], *Marc Pybus*[1], *Gemma Bullich*[2,1], *Mónica Furlano*[1], *Laia Ejarque-Vila*[1], *Laura Lorente Grandoso*[1], *Patricia Ruiz*[1], *Gloria Fraga*[3], *Mercedes López González*[4], *Juan Alberto Piñero Fernández*[5], *Lidia Rodríguez Peña*[5], *Isabel Llano Rivas*[6], *Raquel Sáez*[7], *Anna Bujons-Tur*[1], *Gema Ariceta*[4], *Lluis Guirado*[1], *Roser Torra*[1], *Elisabet Ars*[1]

[1]*Fundació Puigvert, Barcelona, Spain,* [2]*Centre Nacional d'Anàlisi Genòmica (CNAG)-Centre for Genomic Regulation (CRG), Barcelona, Spain,* [3]*Hospital de la Santa Creu i Sant Pau, Barcelona, Spain,* [4]*Hospital Vall d'Hebron, Barcelona, Spain,* [5]*Hospital Universitario Virgen de la Arrixaca, Murcia, Spain,* [6]*Hospital Universitario Cruces, Barakaldo-Bizkaia, Spain,* [7]*Hospital Donostia, San Sebastian, Spain.*

Background: Inherited kidney diseases are one of the leading causes of chronic kidney disease (CKD) that manifests before the age of 30 years. Precise clinical diagnosis of early-onset CKD is complicated due to the high phenotypic overlap, but genetic testing is a powerful diagnostic tool. We aimed to develop a genetic testing strategy to maximize the diagnostic yield for patients presenting with early-onset CKD and to determine the prevalence of the main causative genes.

Methods: We performed genetic testing of 460 patients with early-onset CKD of suspected monogenic cause using next-generation sequencing of a custom-designed kidney disease gene panel in addition to targeted screening for c.428dupC *MUC1*.

Results: We achieved a global diagnostic yield of 65% (300/460), which varied depending on the clinical diagnostic group. Among the 300 genetically diagnosed patients, the clinical diagnosis was confirmed in 77%, a specific diagnosis within a clinical diagnostic group was identified in 15%, and 7% of cases were reclassified. Of the 64 causative genes identified in our cohort, seven (*COL4A3, COL4A4, COL4A5, HNF1B, PKD1, PKD2*, and *PKHD1*) accounted for 66% (198/300) of the genetically diagnosed patients.

Conclusions: Two-thirds of patients with early-onset CKD in this cohort had a genetic cause. Just seven genes were responsible for the majority of diagnoses. Establishing a genetic diagnosis is crucial to define the precise etiology of CKD, which allows accurate genetic counseling and improved patient management.

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P03.026.B iPSC as a genetic model for investigating NAFLD risk variants

Antonio Munoz[1], *Yu-Lin Kuang*[1], *Gilbert Nalula*[1], *Elizabeth Theusch*[1], *Gabriela Sanchez*[2], *Carlos Iribarren*[2], *Ronald M. Krauss*[1], *Aras N. Mattis*[3], *Marisa W. Medina*[1]

[1]*Department of Pediatrics, University of California San Francisco, San Francisco, CA, USA,* [2]*Division of Research, Kaiser Permanente Northern California, Oakland, CA, USA,* [3]*Department of Pathology, University of California San Francisco, San Francisco, CA, USA.*

Introduction: Current cellular genetic models for Non-Alcoholic Fatty Liver Disease (NAFLD) have limitations that prevent the scalable investigation of gene variants. Missense variants rs738409 (I148M) in *PNPLA3* (palatin like phospholipase domain containing protein 3) and rs58542926 (E167K) in *TM6SF2* (transmembrane 6 superfamily member 2) increase hepatocyte intracellular lipid accumulation and are robustly associated with NAFLD. The goal of this study was to assess whether undifferentiated induced pluripotent stem cells (iPSCs) can be used to model the effect of these two NAFLD variants on intracellular lipid accumulation.

Materials and Methods: *PNPLA3* and *TM6SF2* expression were confirmed in iPSCs from individuals of European ancestry carrying *PNPLA3* and/or *TM6SF2* variant alleles ($n = 10$) and non-carriers (n

= 10). Cells were exposed to oleate (0-100μM) or BSA control for 24hrs, and neutral lipids were stained with Nile red, visualized by fluorescence microscopy, and quantified by FACS.

Results: Oleate incubation led to a dose-dependent increase in intracellular lipids, rising 40 ± 18% (mean ± SD) in the oleate-treated cells compared to control-treated cells ($p = 0.001$, $n = 20$). Notably, the increase was greater in *TM6SF2* and/or *PNPLA3* variant carriers (48 ± 16%) compared to non-carriers (24 ± 8 %), $p = 0.002$. Even in BSA-treated cells, there was a trend of elevated lipid levels in carriers compared to non-carriers ($p = 0.25$).

Conclusion: Greater oleate-induced increases of intracellular lipids in iPSCs with the NAFLD risk alleles indicate that undifferentiated iPSCs are a reasonable, more efficient substitute for hepatocytes when studying cellular lipid accumulation and an informative cellular model for the identification and functionalization of NAFLD genetic risk variants. **Funding Sources:** P50GM115318, R01HL139902, P30DK026743

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P03.028.D Genetic heterogeneity of megacystis-microcolon-intestinal hypoperistalsis syndrome

Martin Schwarz[1], *Marcela Malíková*[1], *Júlia Martinková*[1], *Petra Cibulková*[2], *Renáta Michalovská*[3], *Markéta Havlovicová*[1], *Milan Macek, jr*[1]

[1]*Department of Biology and Medical Genetics, Praha, Czech Republic,* [2]*Agel Laboratories - Laboratoře AGEL a.s., Nový Jičín, Czech Republic,* [3]*GHC Genetics s.r.o., Praha, Czech Republic.*

Introduction: Megacystis-microcolon-intestinal hypoperistalsis syndrome (MMIHS; MIM 249210) is a rare multisystem syndrome where its name reflects most commonly affected organs. Although *ACTG2, LMOD1, MYH11, MYL9, MYLK* have been associated with the disease, 55% of all cases remain undiagnosed by genetic testing. Hereby, we report two additional cases with pathogenic variants in *ACTG2*, and a case where a pathogenic variant in *KCNMA1* was found.

Materials and Methods: Next generation sequencing (NGS) was performed. Sanger sequencing was used to verify NGS results and determine the segregation of the presumably pathogenic variants in available first degree relatives.

Results: Two previously described *de novo* heterozygous pathogenic variants in the *ACTG2 /*(c.119G>A p.(Arg40His) and c.770G>A (p.Arg257His)/ where detected. A *de novo* heterozygous Class 4 variant c.1132G>A (p.Gly375Arg) was detected in the *KCNMA1* gene.

Conclusions: Only 47 patients with *ACTG2* related MMIHS have been reported thus far. We identified another two cases which will contribute to the better understanding of this heterogeneous disorder. Variants in *KCNMA1* have also been associated with Liang-Wang multiple malformation syndrome (LIWAS; MIM:618729), where available literature is unfortunately sparse. Previously reported cases with LIWAS bearing the same variant, have among other pathognomonic features also megacystis and impaired bowel motility. Clinical presentation of LIWAS in the neonatal period could be similar to MMIHS with bowel and urinary bladder problems being dominant clinical signs. Therefore, LIWAS should be considered in the differential diagnosis of MMIHS, in particular during early childhood.

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P03.030.B Discovery of novel miRNA markers for pituitary neuroendocrine tumour

Helvijs Niedra[1], *Raitis Peculis*[1], *Ilze Konrade*[2,3], *Inga Balcere*[2,3], *Mihails Romanovs*[2,3], *Liva Steina*[4], *Janis Stukens*[4], *Jelizaveta Sokolovska*[5], *Janis Klovins*[1], ***Vita Rovite***[1]

[1]*Latvian Biomedical Research and Study centre, Riga, Latvia,* [2]*Riga East Clinical University Hospital, Riga, Latvia,* [3]*Riga Stradins University, Riga, Latvia,* [4]*Pauls Stradins Clinical University Hospital, Riga, Latvia,* [5]*University of Latvia Faculty of Medicine, Riga, Latvia.*

Introduction: Recently plasma miRNAs have been studied as biomarkers in various tumors including pituitary neuroendocrine tumors (PitNETs). The identification of PitNET derived miRNAs in plasma remains challenging due to tumor volume and miRNA dilution within blood. To our knowledge this is the first study that used the NGS approach to characterize circulating miRNAs in plasma acquired from Bilateral petrosal sinus sampling (BIPSS) - a gold standard in diagnosis of ACTH secreting PitNETs.

Materials and Methods: We sequenced plasma miRNA samples acquired from sinistral and dextral sides of sinus petrosus inferior and complementary plasma from peripheral blood (before CRH administration, 5 and 15 minutes after stimulation).

Results: The highest amount of differentially expressed miRNAs was observed 5 minutes after CRH stimulation (20 upregulated, 14 downregulated). The highest amount of ACTH was released in the sinistral side during the 5th minute after the stimulation by CRH. In the plasma of sinistral side at the 5th minute we identified two miRNAs: hsa-miR-7-5p and hsa-miR-375-3p that were highly upregulated compared to peripheral blood. Using clustering analysis, we were able to distinguish plasma samples acquired from BIPSS and blood plasma, indicating that the miRNA fraction characterized in this study has potentially PitNET borne origin.

Conclusions: Here we provide the first insight into the landscape of circulating miRNAs in close proximity to PitNET. The data indicates that ACTH secreting PitNET releases two circulating miRNAs upon stimulation with CRH (hsa-mir-7-5p, hsa-mir-375-3p) alongside with ACTH implying the further studies of these miRNA as diagnostic markers.

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P03.031.C *GCK, HNF1A* and *HNF4A* variant analysis in suspected MODY patients

Zivile Zemeckiene*, Marius Sukys, Rimvydas Jonikas, Inga Nasvytiene, Inga Valanciute, Inga Poceviciene, Rasa Ugenskiene*

The Hospital of Lithuanian University of Health Sciences Kauno Klinikos, Kaunas, Lithuania.

Introduction: Maturity-onset diabetes of the young (MODY) is an inherited form of diabetes mellitus which is caused by pathogenic variants in one of 14 known genes, mostly in *GCK, HNF1A* and *HNF4A*. A correct MODY diagnosis might change the treatment and reduce the risk of diabetic complications. In this study we analysed *GCK, HNF1A* and *HNF4A* genes in Lithuanian patients,

consulted for MODY in The Hospital of Lithuanian University of Health Sciences Kauno Klinikos.

Materials and Methods: We performed Sanger sequencing of *GCK*, *HNF1A* and *HNF4A* genes for 77 adults (over 18 years) with suspected MODY during 2018-2020.

Results: For nine patients causative/likely causative variants were determined. Four patients had heterozygous variants previously reported as pathogenic (NM_000162.3(*GCK*):c.234C>G, NM_000162.3*(GCK)*:c.703A>G (two patients), NM_000545.6 (*HNF1A*):c.493T>C). The other variants were novel. Heterozygous *GCK* variant c.471_473del was detected in two patients from the same family with persistent hyperglycaemia. Variant causes deletion of amino acid (p.Glu157del) located in an important enzyme domain. Duplication of 19 nucleotides c.1745_1763dup in *HNF1A* was identified in patient with previously diagnosed diabetes. This variant creates frameshift with premature termination codon (p.Thr589ProfsTer66) which is known mechanism of MODY. Heterozygous *HNF4A* variant c.704G>T was found in two unrelated patients with previously diagnosed diabetes and family history. This variant changes amino acid (p.Arg235Ile) in the conservative ligand binding protein domain and might affect protein structure. All three novel variants were evaluated as likely pathogenic.

Conclusions: Six different pathogenic/likely pathogenic variants were determined in nine patients with suspected MODY diabetes. Three of the variants were novel.

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P03.033.A Clinical description of a new type of mucopolysaccharidosis in Yakutia

Saina Novgorodova[1], *Aytalina Sukhomyasova*[1], *Elizabeth Gurinova*[2], *Vera Argunova*[2], *Lena Nikolaeva*[3], *Nadezhda Maximova*[1]

[1]*Research Laboratory "Molecular Medicine and Human Genetics" of the Medical Institute NEFU, Yakutsk, Russian Federation,* [2]*Republican hospital №1-National Center of Medicine, Yakutsk, Russian Federation,* [3]*Republican Hospital No. 1-National Center of Medicine, Yakutsk, Russian Federation.*

Since 2005, clinical symptoms associated with mucopolysaccharidosis-plus have been recorded in Yakut patients; previously undescribed storage disease with an autosomal-recessive type of inheritance was suspected [Gurinova E.E. 2014]. In 2017, a molecular genetic cause of a new rare autosomal-recessive syndrome in the Yakut and Turkish populations was identified, clinically similar to other types of mucopolysaccharidosis, but has other features of the course, such as congenital heart defects, renal and hematopoietic disorders leading to infant mortality [Kondo et al., 2017; Dursun et al., 2017; Vasilev et al., 2020]. The new disease was added into the McCusick international database under the OMIM number # 617303 and named mucopolysaccharidosis-plus syndrome (MPS-PS). In the Yakut and Turkish populations, the same mutation was found in the VPS33A gene (NM_022916.4: c.1492C> T, NP_075067.2: p. Arg498Trp, subsequently referred to as p.R498W)]. In this thesis, we present brief clinical features of 17 patients with MPS-PS in Yakutia. All children were born in Yakut families from young parents, not in consanguineous marriage. 9 children were born from the second pregnancy and later pregnancies. In 4 cases, pregnancy was complicated by the miscarriage threat; according to ultrasound data, no gestation pathology was detected. A characteristic feature of MPS-PS is early debut and early infant mortality, multisystem organ damage - lungs, kidneys (secondary nephrotic syndrome, severe proteinuria 2-3 g/day, nephromegaly), heart (septal heart disease, severe course), central nervous system and hematopoietic disorders (severe anemia requiring blood transfusion, coagulopathy with hemorrhagic syndrome), the activity of lysosomal hydrolases and urinary glucosaminoglycans test are uninformative.

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P03.035.C A clinical case of patient with cholestatic liver disease due to mutations of the *MYO5B*

Tamara Yurievna Lesnichenko[1], *Natalia Alexandrovna Semenova*[2], *Olga Nikolaevna Ivanova*[2], *Elena Anatolievna Kamenec*[2], *Ekaterina Yurievna Zakharova*[2]

[1]*Federal State Budgetary Educational Institution of Further Professional Education "Russian Medical Academy of Continuous Professional Education" of the Ministry of Healthcare of the Russian Federation, Moscow, Russian Federation,* [2]*Federal State Budgetary Scientific Institution "Research Centre for Medical Genetics", Moscow, Russian Federation.*

Introduction: Defects in *MYO5B* gene, on which bile salt export pump depends to localize to the canalicular hepatocellular membrane usually cause diarrhea 2, with microvillus atrophy (OMIM # 251850) but also may result in liver disease without enteropathy. It characterized by low-GGT-cholestasis variable severity, hepatomegaly, normal or mildly elevated transaminases.

Materials and Methods: We present a female patient at the age of 2y4m with isolated cholestasis. Her infantile period was normal. The disease manifested by high temperature, hepatomegaly, acholic stools. Serum clinical laboratory tests showed cholestasis with normal GGT activity, conjugated hyperbilirubinemia 30mmol/l, normal transaminase levels, increased serum alkaline phosphatase 587E/l, hypercholesterolemia 8,03mmol/l. Deficiency of lysosomal acid lipase was excluded by an enzyme test. NGS was used to analyze 52 genes responsible for hereditary diseases with cholestasis. Two nucleotide variants in *MYO5B* gene were detected.

Results: We identified a novel heterozygous frameshift duplication c.1604_1610dupGCCAGCA p.His537GlnfsTer28. Another heterozygous variant c.1201C>T (p.Arg401Cys rs761492029), revealed in the *MYO5B* gene, described as pathogenic. Then, we performed Sanger sequencing on the DNA extracted from the mother, the father and the sister of the proband to analyze the segregation of each genetic variant identified by NGS. Parents and older sibling are healthy and heterozygous for one or another mutant allele.

Conclusions: Thus, the findings support the disease-causing role of novel *MYO5B* mutation. Many patients with low-GGT-cholestasis remain genetically undiagnosed. Verification of the genetic aetiology of cholestasis allows selection of appropriate treatment strategies, determination of genetic risk and discussion of the necessity of prenatal testing.

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P03.036.D Identification of gene variants in Indonesian Hirschsprung disease patients using whole exome sequencing

Gunadi[1], *Alvin Santoso Kalim*[1], *Marcellus*[1], *Dyah Ayu Puspitarani*[1], *Kristy Iskandar*[2], *Galuh Dyah Nur Astuti*[3,4]

[1]Pediatric Surgery Division, Department of Surgery/Genetics Working Group, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada/Dr. Sardjito Hospital, Yogyakarta, Indonesia, [2]Department of Child Health/Genetics Working Group, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada/Dr. Sardjito Hospital, Yogyakarta, Indonesia, [3]Department of Human Genetics, Radboud University Medical Center, Nijmegen, Netherlands, [4]Division of Human Genetics, Center for Biomedical Research, Faculty of Medicine, Diponegoro University, Semarang, Indonesia.

Introduction: Hirschsprung's disease (HSCR) is a complex genetic disorder with at least 24 genes have been identified for the pathogenesis of HSCR. However, they only contributed to 20% of HSCR cases. We aimed to further elucidate the genetic basis of HSCR in Indonesia.

Materials and Methods: Whole exome sequencing was performed in 20 sporadic isolated HSCR patients and four non-HSCR subjects as controls. We excluded variants presented in controls, followed by *in silico* prediction tools and population allele frequency databases to select rare variants. We determined the minor allele frequency (MAF) using gnomAD (MAF < 0.1%).

Results: We involved 11 (55%) males and 9 (45%) females. We identified several candidate genes, including *PTPN13, UBR4, ECE1, GDNDF, ASTN1, CELSR3, XYLT1, SORL1* and *FAT1*. One of them, a novel frameshift variant in *PTPN13* gene (c.5763delT; p.Tyr1921Ter) which may lead to loss of function on protein level. Moreover, we also identified compound heterozygous variants in *MUTYH* gene: the first variant, a known protein truncating variant associated with colorectal cancer, c.1354G>T; p.Glu452* and the second variant is a novel c.116C>T; p.Ala39Val variant. We also found co-mutation of *UBR4* gene (c.5497G>A; p.Gly1833Arg) and novel variant of *GDNF* gene (c.349G>A; p.Gly117Ser) in one patient.

Conclusions: We identified several novel genes and variants that might contribute to the pathogenesis of HSCR. Our study further confirms the complex network during the enteric nervous system development and HSCR pathogenesis. This study was funded by the World Class Research grant (Ministry of Research and Technology/National Research and Innovation Agency, Indonesia

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P03.037.A A case of familial brain-lung-thyroid syndrome due to a *NKX2.1* run-on mutation

*Federica Baldan[1], Elena Cavaliere[1,2], Anna J. Gortan[1,2], Nadia Passon[2], Dora Fabbro[2], Dario Marin[1,2], Myriam Carecchio[3], Sara C. Credendino[4], Rosa Gallo[4], Paola Cogo[1,2], **Giuseppe Damante**[1,2], Gabriella De Vita[4]*

[1]University of Udine, Udine, Italy, [2]Academic Hospital of Udine, Udine, Italy, [3]University of Padua, Padua, Italy, [4]University of Naples, Udine, Italy.

NKX2.1 is a homeobox gene encoding a tissue-specific transcription factor able to bind DNA by its homeodomain. NKX2.1 expression is fundamental for thyroid, lung and ventral forebrain development and correct maturation. Indeed, loss of function mutations of this gene cause the 'brain-lung-thyroid' syndrome (BLTs), which is characterized by various combinations of thyroid dysgenesis, infant respiratory distress syndrome, and hyperkinetic movement disorders, mainly chorea. Here, we describe a familial case of BLTs due to a NKX2.1 run-on mutation. The proband is an eleven-year-old boy who, at birth, suffered from respiratory distress associated to pulmonary hypertension. Delayed motor and speech language milestones were reported. Moreover, he suffered from frequent upper-respiratory infections, short stature due to subclinical hypothyroidism, sleep disturbance and learning difficulties. A neurologic assessment revealed generalized chorea unstable gait and mild hypotonia. The patient's mother suffered of impaired gait and frequents falls, as well as learning disability and subclinical hypothyroidism. On examination, she presented with distal choreiform movements in the lower limbs. The patient's aunt and grandmother reported gait imbalance and frequents falls during childhood; none of them presented with movement disorder. Genetic testing for the NKX2.1 gene was performed and a run-on mutation (c.1204dupT; p.*402Leuext*37) was identified: duplication of the thymine corresponding to the first base of the stop codon causes the changing of the stop codon in a leucine-coding codon. The resulting frameshift generated a tail of 36 additional amino acids at the protein C-terminus. Segregation analysis confirmed that all the affected relatives carried the same genetic variant.

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P03.038.B Effects of growth hormone treatment in Noonan syndrome: correlations with genotype

***Diana Miclea**[1,2], Yline Capri[2], Alain Verloes[2]*

[1]"Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Cluj-Napoca, Romania, [2]Department of Genetics, APHP-Robert Debré University Hospital, Paris, France.

Background: In Noonan syndrome(NS), the treatment with growth hormone(GH) has been carried out in the last decades, with reported effects on improvement of growth velocity and adult height, however, these data usually included patients with NS phenotype, only the last few years bringing data about GH treatment in genotyped NS patients.**Aim**.To evaluate the effect of GH treatment according to genotype in patients with NS, by a systematic review of the literature.

Methods: We evaluated auxological, hormonal and imagistic data before and under GH treatment in patients with genetically confirmed NS reported in literature between 2001 and 2020.

Results: We identified 21 studies, including data from 336 genotyped patients with NS. Of these, 243patients(72.3%) presented pathogenic variant for *PTPN11*gene, 17patients(5%) for *SOS1*, 15 patients(4.4%) for *RAF1*, 9patients(2.6%) for *SHOC2*, 8patients(2.3%) for *KRAS*, 5patients(1.4%) for BRAF and 2patients (0.5%) for each of the genes *MEK1,SPRED1,HRAS,NRAS* and *RIT1*. The age of GH treatment initiation was 8.3years. The mean period of treatment was of 6.7years. The height before GH treatment was -2.8SD and at the end of GH treatment was of -2.1SD, with a mean adult height of 165.6cm for males and 155.4cm for females. The target height was -0.6SD. It was not observed a difference on growth between the patient categories according to the genes.

Conclusion: This study is the first review about the pattern of growth under GH treatment in genotyped NS and even it was thought that this pattern is well known, we need more data to conclude the real effect on growth regarding each gene involved in NS.

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P03.039.C Next generation sequencing in the diagnostic approach to autosomal dominant polycystic kidney disease

***Deimante Brazdziunaite**[1], Marius Miglinas[2], Algirdas Utkus[1]*

[1]Department of Human and Medical Genetics, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, Vilnius, Lithuania, [2]Center of Nephrology, Institute of Clinical Medicine, Faculty of Medicine, Vilnius University, Vilnius, Lithuania.

Introduction: Classically, autosomal dominant polycystic kidney disease (ADPKD) is presented as a common Mendelian disorder and represent the leading genetic causes of renal disease in adults. It is caused by mutations either in *PKD1* or *PKD2*. However, next-generation sequencing (NGS) offers the opportunity to significantly improve the diagnostic yield in ADPKD. **Body**. Introduction of the first therapy approved for ADPKD - vasopressin V2-receptor antagonist (Tolvaptan) - brought more new attention to this disease. Using European Renal Association-European Dialysis and Transplant Association Registry, and population based studies, Willey et al. (2016) indicated that the ADPKD point prevalence in the EU is <5/10,000. Moreover, disease appears to be quite genetically heterogeneous. The PKD mutation database counts more than 1000 pathogenic variants in *PKD1* and about 200 in *PKD2* gene. According to various authors, about 8-10% of ADPKD cases do not have pathogenic variant in *PKD1/2* genes. Possibility to apply extended diagnostics by NGS revealed previously unknown causes. Pathogenic variants in *GANAB* gene and PKD phenocopies due to variants in *HNF1β*, *PKHD1*, *DNAJB11*, or *TSC1/2* genes are described. However, the list of novel phenocopies and potential candidate genes is not final yet. This brings questions whether clinical diagnostic criteria will be as specific and sensitive in distinguishing these new genetic forms and phenocopies of PKD.

Conclusion: Technological advances let us distinguish different aetiologies of apparently the same disease as well as expand our knowledge. Furthermore, in the era of individualised treatment the most accurate molecular diagnosis is required.

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P03.041.A Protein loosing enteropathy and lymphedema in a girl with a homozygous DCHS1 mutation

*Tatiana V. Gabrusskaya[1], **Evgeny N. Suspitsin**[1,2], Elizaveta Y. Lapina[1], Elena A. Kornienko[1]*

[1]St.-Petersburg State Pediatric Medical University, St.-Petersburg, Russian Federation, [2]N.N. Petrov Institute of Oncology, St.-Petersburg, Russian Federation.

Introduction: Hennekam syndrome (HS) and van Maldergem syndrome (VMS) are two entities sharing some clinical features. While unusual facial gestalt and some degree of developmental delay is observed in both conditions intestinal lymphangiectasia and lymphedema have been considered exclusive for HS.

Materials and Methods: We present a case of 5 year-old girl of Syrian descent with severe protein loosing enteropathy (PLE) due to lymphangiectasia, lymphatic malformation in mediastinum, asymmetric lymphedema, facial dysmorphisms (flat nasal bridge, epicanthus, microtia), bilateral hearing loss and growth retardation. No parental consanguinity was reported. She was born preterm, had severe course of bronchopulmonary dysplasia and developed symptoms of PLE in the first year of life. The girl had hypoproteinemia, hypoalbuminemia, and elevated fecal alpha-1 antitrypsin. Right arm and left leg lymphedema became obvious by the age of 5. The patient was suspected to have Hennekam syndrome and subjected to clinical exome sequencing.

Results: No pathogenic variants in Hennekam syndrome-associated genes (CD55, FAT4, CCBE1 and ADAMTS3) have been found. Rare DCHS1 variant c.1954A>G (p.S652G) was detected in homozygous state; biallelic DCHS1 alterations have been previously described in some patients with van Maldergem syndrome type 1.

Conclusion: Since DCHS1 and FAT4 molecules form a receptor - ligand pair, we hypothesize that DCHS1 mutation may cause HS-like phenotype. We demonstrate that lymphatic pathology including PLE, lymphatic malformation and lymphedema can be a part of both conditions. This observation extends existing data on clinical overlap between VMS and HS.

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P03.042.B Male pseudo hermaphroditism in a 54 years old woman

María Luz Bellido[1], *Teresa de Haro[1], Trinidad Gonzalez[2], Matias Perez[1]*

[1]Hospital Universitario Virgen de las Nieves, Granada, Spain, [2]Hospital Universitario Clínico San Cecilio, Granada, Spain.

Introduction: Disorders of sex development (DSD) comprise a heterogeneous group of congenital conditions associated with atypical development of internal and external genitalia. Sex determination is governed by genes from SRY region at Y chromosome that initiates the male developmental program. However, there exist many other genes involve in this process.We describe the case of a 54-years-old woman with an incidental finding of absence of uterus, ovaries, prostate or seminal vesicles, after a TC scan performed for other pathologies.

Methods: GTG-banding karyotype was performed according to standard protocols. Twenty metaphases chromosomes were examined.Next-generation sequencing study was done by MiSeq analyzer from Illumina®.

Results: Karyotype result: male, 46,XY.Sequencing study finding:

Gene (localization)	Variant	Type	Status	dbSNP/ HGMD	Heritage	Classification
SRD5A2 (2p23.1)	c.679C>T, (p. Arg227Ter)	Nonsense	Heterozygous	rs121434248 / CM920642	AR	Pathogenic
	c.344G>A, (p. Gly115Asp)	Missense	Heterozygous	rs121434246 / CM920633	AR	Probably Pathogenic

SRD5A2 codifies to 5-alpha-reductase type 2. This enzyme catalyzes the conversion of testosterone to dihydrotestosterone, which is essential for normal differentiation of the external male genitalia and virilization.

Conclusion: The final diagnosis was male pseudo-hermaphroditism due to 5-alpha-reductase type 2 deficiency (OMIM#264600). 46,XY patients show ambiguous genitalia at birth, including perineal hypospadias and a persistent urogenital sinus with a blind perineal vaginal orifice.DSD used to be diagnosed at birth or childhood by pediatricians or within the family, making our case especially unusual. The patients need an individualized management, especially for decisions related to sex of rearing, future intervention, hormone treatment and reproductive options.

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P03.044.D The importance of NGS data reanalysis and further functional analysis: an example of impaired phosphate metabolism

Margarita Sharova[1], *Svetlana Papizh[2], Olga Levchenko[1], Alexandra Filatova[1], Andrey Marakhonov[1], Anatoliy Tulpakov[1], Mikhail Skoblov[1]*

[1]Research Centre for Medical Genetics, Moscow, Russian Federation, [2]Veltischev Research and Clinical Institute for Pediatrics of the Pirogov Russian National Research Medical University, Moscow, Russian Federation.

Introduction: The regulation of phosphate metabolism occurs mainly in kidneys through reabsorption of phosphate ions by the NaPi-IIa (SLC34A1) and NaPi-IIc (SLC34A3) transporters. Pathogenic variants in these genes are identified in patients with hereditary hypophosphatemic rickets with hypercalciuria or hypercalcemia, infantile 2.

Materials and methods: patients with a clinical diagnosis of hereditary hypophosphatemic rickets were referred for molecular genetic testing. Three patients underwent full-genome sequencing followed by reanalysis of genomic data; for one NGS panel data were reanalyzed. Experimental validation of the effect of variants on splicing was performed using minigene and RNA analysis.

Results: Primary NGS data analysis didn't reveal causative variants for the phenotype or there was only one variant for autosomal recessive disease. Further investigation of data found in-frame deletion p.91_97del in SLC34A1 gene with global frequency 1,7% in two patients in compound heterozygous state, that previously was described as pathogenic. Intronic pathogenic deletion c.925+20_926-48del101was found in two brothers in compound heterozygous state. Also, VUS c.1449G>A in SLC34A1 gene was detected. Minigene assay showed that the variant leads to truncation of exon 13 by 34 nucleotides with frameshift and PTC formation. Another functional study by RT-PCR identified variant c.846G>A in SLC34A3 as splicing variant, leading to skipping of exon 8 without frameshift.

Conclusion: Reanalysis of genomic data is important not only over time, but also by other clinical bioinformaticians. Some pathogenic variants with high global frequency could be filtered out in the early stages of NGS analysis. Functional analysis allows to investigate the pathogenicity of VUS.

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P03.045.A Contribution of a non-coding variant in autosomal recessive ACTG2-related visceral myopathy identified by whole-genome sequencing

Mari Mori[1,2], *Kristen V. Truxal*[1,2], *R. Tanner Hagelstrom*[3], *Amanda Clause*[4], *Vinay Prasad*[5,2], *Kandamurugu Manickam*[1,2], *Stephen G. Kaler*[1,2,6], *Maria M. Alves*[7], *Carlo Di Lorenzo*[8,2]

[1]Division of Genetic and Genomic Medicine, Nationwide Children's Hospital, Columbus, OH, USA, [2]Department of Pediatrics, The Ohio State University, Columbus, OH, USA, [3]Illumina Inc, San Diego, CA, USA, [4]Nationwide Children's Hospital, Columbus, OH, USA, [5]Pathology & Laboratory Medicine, Nationwide Children's Hospital, Columbus, OH, USA, [6]Center for Gene Therapy, Abigail Wexner Research Institute, Nationwide Children's Hospital, Columbus, OH, USA, [7]Department of Clinical Genetics, Erasmus University Medical Center, Rotterdam, Netherlands, [8]Division of Pediatric Gastroenterology, Hepatology and Nutrition, Nationwide Children's Hospital, Columbus, OH, USA.

Introduction: Pediatric intestinal pseudo-obstruction (PIPO) is a heterogeneous condition characterized by impaired gastrointestinal propulsion and a broad clinical spectrum ranging from a severe form requiring parenteral nutrition to milder forms with some ability to tolerate oral nutrition. Various molecular bases underlying primary PIPO have been identified, of which ACTG2-related visceral myopathy is the most common for familial or sporadic (de novo) primary PIPO with autosomal dominant inheritance. However, a recent case report suggests that some mild ACTG2 variants may be associated with autosomal recessive inheritance. We present a family in which both parents have relatively mild gastrointestinal symptoms, and two sons have severe PIPO, consistent with autosomal recessive inheritance.

Results: Whole-genome sequencing (WGS) revealed a missense variant c.28G>A (p.Val10Met) in the ACTG2 gene in the mother and the sons, and deletion of ACTG2 exon 1 (non-coding) in the sons and presumably in the father.

Conclusions: Our case reinforces that monoallelic mild ACTG2 variants may underly mild primary PIPO, while biallelic mild variants can cause severe diseases. Prior molecular studies on PIPO in the literature were based on whole-exome sequencing or targeted Sanger sequencing, which may fail to detect deletion of the non-coding exon. Alterations in non-coding ACTG2 segments can be under-recognized causes of mild gastrointestinal symptoms and may explain some instances of inter-familial variability. WGS offers a more comprehensive genetic workup for severe primary or idiopathic PIPO because of such genetic heterogeneity. Notably, the absence of family history does not reliably exclude the presence of genetic causes in PIPO.

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P03.046.B Application of NGS sequencing for improved diagnosis in the pediatric nephrology setting

Radosveta Bozhilova[1], *Olga Beltcheva*[1], *Galia Zlatanova*[2], *Kunka Kamenarova*[1], *Kalina Mihova*[1], *Felitsiya Shakova*[1], *Dimitar Roussinov*[2], *Maria Gaydarova*[2], *Vanio Mitev*[1], *Radka Kaneva*[1]

[1]Molecular Medicine Center, Dpt. Medical Chemistry and Biochemistry, Medical University – Sofia, Sofia, Bulgaria, Sofia, Bulgaria, [2]SBAL Pediatric Diseases, Nephrology and Hemodialysis Clinic, Department of Pediatrics, Medical University -Sofia, Sofia, Bulgaria, Sofia, Bulgaria.

Introduction: Nephrotic syndrome, resulting from pathological changes in the glomerular filter, is a common childhood disorder. While it usually resolves with corticosteroid treatment, 10-20% of the cases are resistant to therapy and often progress to end-stage renal disease. A monogenic cause of steroid resistant nephrotic syndrome (SRNS) is identified in up to half of the cases and more than 50 genes have been associated with the disease.

Materials and Methods: Nine SRNS patients, initially screened for *NPHS1*, *NPHS2* and *WT1* mutations, were recruited. Two children presented with extrarenal features - seizures and brain infarctions. We used TruSight One Sequencing Panel (Illumina) on MiSeq platform for identification of disease-causing variants and Sanger sequencing for confirming the mutations and establishing

their origin. The pathogenicity of novel variants was evaluated based on the ACMG criteria.

Results: Homozygous mutation in *NOS1AP*, recently found to be associated with SRNS, was identified in one case. An additional rare *MYH11* variant may account for the brain infarctions in this child. A heterozygous frameshift mutation in *CFHR5* and combinations of rare amino acid substitutions in known SRNS genes (*ACTN4*, *LAMB2*, etc.) accounted for three additional cases.

Conclusion: The application of NGS for screening of an extended gene panel allowed us to identify the genetic cause of the disease in approximately half of the SRNS patients recruited for the study. As expected, a wide variability of genes and mutation types were involved. A possible oligogenic inheritance was observed in some of the cases. Grant references: D-93/24.06.2020; D01-285/17.12.2019, MES, Bulgaria

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P03.047.C Functional consequences of rs1800629 *TNF* gene associated with asthma and tuberculosis development

Irina Zhalsanova, *Elena Bragina, Nadezhda Babushkina, Nataliya Tarasenko, Maxim Freidin, Valery Puzyrev*

Research Institute of Medical Genetics, Tomsk National Research Medical Center of the Russian Academy of Sciences, Tomsk, Russian Federation.

Introduction: Tumor necrosis factor-alpha (TNF) is one of the proinflammatory cytokines involved in the various infectious and allergic diseases development. We studied the SNP(rs1800629) association located in the *TNF* gene promoter region with bronchial asthma (BA) and tuberculosis (TB) development and evaluated the functional consequences of the rs1800629 by analyzing the expression level in short-term cell cultures of peripheral blood mononuclear cells (PBMC) stimulated by inducers (lipopolysaccharide (LPS), interferon-gamma (IFN-γ)).

Methods: Genotyping was performed using qPCR among patients with BA, TB, and in the control group ($n = 1231$). *TNF* gene expression level was evaluated response to stimulation in PBMC from healthy donors, differentiated on genotypes rs1800629 ($n = 17$). Samples were incubated with stimuli (LPS, IFN-γ), and control samples without stimuli within overnight at +37°C. Target gene expression was analyzed by RT-PCR.

Results: Association analysis showed, AA genotype frequency in TB patients was higher than in patients with BA ($p = 0.047$) and the control group ($p = 0.033$). *TNF* gene expression level upon IFN-γ stimulation was 4,2 times higher compared to unstimulated PBMC ($p < 0.05$). The A allele (AA + GA) presence promoted a 1.5-fold decrease in *TNF* gene expression upon stimulation with IFN-γ compared to GG. *TNF* gene expression increased upon LPS stimulation, but no significantly.

Conclusions: Thus, differences in the *TNF* gene transcription pattern depending on the genotype and the stimulation mode. These features are important for understanding the genetic susceptibility to infectious and allergic diseases of individuals living in regions with different infectious load levels. The study was supported by the RFBR grant No.15-04-05852.

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P03.048.D Genome wide association study of type 2 diabetes complications in population of Latvia

Raitis Peculis[1], *Monta Ustinova*[1], *Raimonds Rescenko*[1], *Vita Rovite*[1], *Linda Zaharenko*[1], *Ilze Elbere*[1], *Laila Silamikele*[1], *Ilze Konrade*[2,1], *Jelizaveta Sokolovska*[3], *Valdis Pirags*[3,1], *Janis Klovins*[1]

[1]*Latvian Biomedical Research and Study Centre, Riga, Latvia,* [2]*Faculty of Medicine, Riga Stradins University, Riga, Latvia,* [3]*Faculty of Medicine, University of Latvia, Riga, Latvia.*

Introduction: Complications of type 2 diabetes affect a significant proportion of patients and their prevalence, correlates with increased duration of diabetes. Yet, time of onset of complications is variable for different persons with type 2 diabetes suggesting an individual level predisposition and protection. Therefore, investigation of the genetic component of type 2 diabetes complications is warranted.

Materials and methods: We have assayed 601 type 2 diabetes patients with four common diabetes complications using Human GSA genotyping array. Genome wide association study was performed to investigate the genetic background of following phenotypes: diabetic neuropathy, diabetic nephropathy, macrovascular events, and ophthalmic complications. Genotype analysis was performed comparing diabetes patients with complication present to those without particular complication.

Results: Association analysis identified eight novel type 2 diabetes susceptibility loci with genome wide significance. Two SNPs, rs1132787 (*GYPA*) and rs522521 (*LOC105371557*) were associated with diabetic neuropathy, while seven (one shared with diabetic neuropathy) rs2477088 (*PDE4DIP*), rs522521 (*LOC105371557*), rs4852954 (*NAT8*), rs6032 (*F5*), rs6935464 (*RPS6KA2*), rs7236163 (*ZNF519*), and rs3095447 (*CCDC146*) (latter is also shared with ophthalmic complications) were showed significant association with macrovascular complications. The association results were adjusted for covariates (age, sex, BMI, diabetes duration, median HbA1c, and prescription drug use) and a genome-wide significant threshold of $P < 5 \times 10^{-8}$ was used.

Conclusions: Using the genome-wide genotyping approach this study identified ten novel associations with T2DM complications (at eight loci) and provides additional insight into genetic markers of these phenotypes. The study was supported by European Regional Development Fund Project No 1.1.1.2/VIAA/2/18/287.

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P04 Skeletal, Connective Tissue, Ectodermal and Skin Disorders

P04.001.B 6q13 microdeletion - mapping the clinical phenotype

Raquel Rodrigues, *Oana Moldovan, Rosário Silveira-Santos, Ana B. Sousa, Ana Sousa*

Hospital de Santa Maria, Lisboa, Portugal.

Introduction: Proximal 6q11q14 deletions are exceedingly rare, particularly the enclosed 6q13q14 microdeletions. Little is known about their phenotypic consequences, which include mild/moderate developmental delay/intellectual disability (DD/ID), minor dysmorphism, and connective tissue abnormalities. We describe a case of 6q13 microdeletion, among the smallest reported within this syndromic region, aiming to contribute to the genomic mapping of associated clinical features.

Methods: The proband, a 34yo male, was referred to the Genetics Department for mild ID and motor clumsiness. In childhood, he presented mild DD, language delay and learning difficulties. Additional features included strabismus, cryptorchidism, joint hypermobility, and abnormal foot position. A DNA sample was analyzed by array-CGH (180K CGX-HD). Segregation was investigated by FISH.

Results: Array-CGH revealed a 4.71Mb microdeletion in 6q13 (70917114_75625720; hg19), encompassing 17 OMIM genes. Parental studies confirmed this deletion occurred *de novo*.

Discussion: Previous reports proposed candidate genes for DD/ID within 6q11q4 region, namely *KCNQ5*, which is deleted in our patient. However, the chief candidate gene underlying learning difficulties and language delay was proposed to be *HRT1B*, which is not deleted in this case. DD/ID is unspecific and frequently features in contiguous deletion syndromes, probably contributed by several genes in the deleted interval. Conversely, connective tissue abnormalities are specific characteristics often observed in 6q11q14 microdeletions, including hypermobility and foot deformities. Thus far, the latter have been assigned to *COL12A1* gene. However, *COL12A1* is not deleted in our case, suggesting a key role for other genes, such as *COL9A1* and *COL19A1*, in the etiology of these problems.

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P04.002.C A novel truncating variant in the *FGD1* gene associated with Aarskog-Scott syndrome in a family previously diagnosed with Tel Hashomer camptodactyly

Lena Sagi-Dain[1], Irena Kessel[1], Amir Peleg[1], Tamar Paperna[2], Nina Ekhilevitch[2], Alina Kurolap[3], Hagit Baris Feldman[3], ***Marina Bar-Shay***[1]

[1]Carmel Medical Center, Haifa, Israel, [2]Rambam Medical Center, Haifa, Israel, [3]Ichilov Medical Center, Tel Aviv, Israel.

Tel Hashomer camptodactyly syndrome is a genetic association, characterized by camptodactyly with muscular hypoplasia, skeletal dysplasia, and abnormal palmar creases. Currently, the genetic basis for this disorder is unknown, thus there is a possibility that this association may be contained within another genetic diagnosis. In this manuscript we present a family with a previous clinical diagnosis of Tel Hashomer camptodactyly syndrome. Whole exome sequencing revealed a novel hemizygous truncating variant c.269_270dup (p.Phe91Alafs*34) in the *FGD1* gene (NM_004463.3) in all three symptomatic patients, congruous with a diagnosis of Aarskog-Scott syndrome. Our manuscript adds to the limited data on Aarskog-Scott syndrome, and emphasizes the importance of unbiased comprehensive genetic testing towards establishing a diagnosis for genetic associations.

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P04.003.D Abdominal wall defects in the Czech Republic: Frequency and prenatal diagnostics

Antonin Sipek Sr[1,2,3], *Vladimir Gregor[1,2], Antonin Sipek Jr[1,4,5], Jan Klaschka[6,7], Marek Maly[6,8], Jitka Jirova[9]*

[1]Department of Medical Genetics, Thomayer University Hospital, Prague 4, Czech Republic, [2]Department of Medical Genetics, Pronatal Sanatorium, Prague, Czech Republic, [3]Department of Medical Genetics, Centre for Medical Genetics and Reproductive Medicine, Gennet, Prague, Czech Republic, [4]Institute of Biology and Medical Genetics, 1st Faculty of Medicine, Charles University, Prague, Czech Republic, [5]Institute of Medical Genetics, 3rd Faculty of Medicine, Charles University, Prague, Czech Republic, [6]Institute of Computer Science of the Czech Academy of Sciences, Prague, Czech Republic, [7]Institute of Biophysics and Informatics, First Faculty of Medicine, Charles University, Prague, Czech Republic, [8]National Institute of Public Health, Prague, Czech Republic, [9]Institute of Health Information and Statistics of the Czech Republic, Prague, Czech Republic.

Introduction: Omphalocele and Gastroschisis are the most important anomalies from the group of Abdominal Wall Defects (AWD). The main goal of our study was to evaluate the changes in the frequency of these anomalies in the Czech Republic and the overall effectiveness of their prenatal diagnostics.

Materials and Methods: We are using the official population data from the National Registry of Congenital Anomalies of the Czech Republic, which is run by the Institue of Health Information and Statistics of the Czech Republic. The registration process is country-wide and compulsory by the national law. We analyzed the numbers of omphalocele (Q792) and gastroschisis (Q793) cases in the newborns and prenatally diagnosed cases in the Czech Republic (1994-2018).

Results: The total incidence of omphalocele was 3.32 per 10.000 live births (1.39 in births and 1.93 in prenatally diagnosed cases). For gastroschisis, the total incidence was 3.09 (0.90 in births and 2.19 in prenatally diagnosed cases). The total frequency of both anomalies increased during the selected period in the Czech Republic, the increase is statically significant ($p < 0.05$). The average week of gestation at the time of positive prenatal diagnostics decreased significantly for both anomalies ($P < 0.05$).

Conclusions: The improvement of ultrasound prenatal diagnostics enabled the successful detection of AWDs in earlier gestation weeks. The overall incidence of both anomalies is however increasing in the Czech Republic, as more and more mothers prefer surgical intervention over the termination of the pregnancy.

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P04.004.A Biallelic deep intronic variants c.1528-126A>G and c.5457+81T>A in *TRIP11* are associated with achondrogenesis 1A

Priyanka Upadhyai*, Radhakrishnan Periyasamy, Vishal S. Guleria, Shalini Nayak, Katta M. Girisha*

Kasturba Medical College, Manipal Academy of Higher Education, Manipal, India.

Introduction: Biallelic loss of function variants in *TRIP11* encoding for GMAP-210 cause lethal chondrodysplasia achondrogenesis type 1A (ACG1A). Complete depletion of *TRIP11* impairs Golgi structure, vesicular transport and results in loss of IFT20 anchorage to the Golgi that is vital for ciliary trafficking and ciliogenesis. Here we report four affected foetuses, two each from two families, homozygous for putative pathogenic deep intronic variants in *TRIP11*.

Materials and Methods: Perinatal autopsy and radiological evaluation was performed, followed by exome and genome sequencing. Segregation analysis was performed by Sanger sequencing. Fibroblast cell-lines were available from an affected foetus. Transcript analysis was performed by qRT-PCR and RT-PCR respectively. Protein levels were ascertained by immunoblotting. The Golgi, ciliogenesis and autophagy were evaluated by immunostaining.

Results: The deep intronic variants c.1528-126A>G and c.5457 +81T>A in *TRIP11* were present in homozygous state in all affected foetuses and the parents were heterozygous carriers. This led to drastic depletion of *TRIP11* mRNA, protein levels and produced severely compacted Golgi in fibroblasts. The c.5457 +81T>A variant caused aberrant splicing and retention of 77 base-pairs of intron 18. We observed severe depletion of ciliated fibroblasts and reduction in cilia length, not reported so far in patient cells with *TRIP11*-related disorder. Moderate autophagic dysfunction was noted in affected fibroblasts, congruent with the role of IFT20 in modulating autophagy in primary cilia dependent manner in some cellular contexts.

Conclusion: Our findings illustrate how pathogenic variants occurring in deep intronic and putative regulatory regions of *TRIP11* may impact its expression and activity leading to ACG1A.

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P04.005.B Pre and post-natal achondroplasia, retrospective series of 64 consecutives cases with analyze of the diagnostic methods and timing issues

Genevieve Baujat, *Roxana Borghese, Pascale Sonigo, Joana Bengoa, Caroline Michot, Elodie Millischer-Bellaiche, Sophie Rondeau, Beatrice Childs, Tania Attie-Bitach, Bettina Bessieres, Laurent Salomon, Yves Ville, Jean-Paul Bonnefont, Julie Steffann, Valerie Cormier-Daire*

Necker Hospital, Paris, France.

The last years, diagnosis of achondroplasia benefited of advances in prenatal imaging, and in invasive and non-invasive molecular screening.

Objectives: To analyse diagnosis procedures and outcome on 64 consecutive confirmed cases of achondroplasia seen in the French Centre of Reference for Skeletal Dysplasia, between 2008 and 2016.MethodsDiagnosis stage/ age, analyse of the achondroplasia features by imaging (ultrasound (US), 3D-CT), molecular confirmation method, and pregnancy outcome were retrospectively determined.

Results: 64 cases of achondroplasia were included. The diagnosis was made during the pregnancy in 43 cases (67%, mean stage of 30 weeks). For the remaining 21 cases (33%), the diagnosis was performed at birth in all cases but one, diagnosed at 2 months. Among the eight foetuses with inherited ACH: 4 were diagnosed after early chorionic villus sampling, leading to termination of pregnancy (TOP) and 4 diagnosed after 26 weeks, by US.In the de novo prenatal 35 cases, mid-trimester US was normal in 80% of cases. The diagnosis was confirmed by the 3D-CT in all 17 cases when performed (52%), and/ or by molecular screening after amniocentesis (43%). The prenatal diagnosis led to TOP in 12 cases (34%), in a mean stage of 32 weeks. 66% of the foetuses diagnosed in prenatal went into the birth.

Conclusion: The systematic screening of the second term was normal in 80%. One third of the diagnosis led to TOP. The results confirm the late diagnosis of de novo achondroplasia during pregnancy, leading to major psychological and ethical issues for the parents.

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P04.007.D Shared Runs of Heterozygosity Mapping using whole genome sequencing reveals a complex structural variant in *GSN* causing novel cutaneous-visceral organ Gelsolin amyloidosis

Adam Jackson[1,2], *Glenda Sobey*[3], *Ashutosh Wechalekar*[4], *Dorota Rowczenio*[4], *. Genomics England Research Consortium*[5], *Glenda Beaman*[2], *Rob McMahon*[1], *Unzela Khan*[1], *Emma Miles*[1], *Amy Goldman*[1], *Simon Lovell*[6], *Jill Clayton-Smith*[1,2], *Siddharth Banka*[1,2]

[1]*Manchester Centre for Genomic Medicine, Manchester, United Kingdom,* [2]*Division of Evolution and Genomic Sciences, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom,* [3]*National EDS Service, Sheffield Children's NHS Foundation Trust, Sheffield, United Kingdom,* [4]*National Amyloidosis Centre, University College London (Royal Free Campus), London, United Kingdom,* [5]*Genomics England, London, United Kingdom,* [6]*Division of Evolution and Genomic Sciences, School of Biological Sciences, University of Manchester, Manchester, United Kingdom.*

Introduction: We report a multi-generational family of several individuals with cutis laxa, bowel perforation, cardiac rupture and clinical diagnosis of Ehlers-Danlos syndrome. Several histopathological, targeted sequencing studies failed to identify the genetic cause. Two affected individuals were detected with cutaneous amyloid, but proteomic studies failed to reveal its origin.

Methods and Results: We devised a novel Heterozygosity Mapping approach using srWGS data and identified a ~10.38Mb disease-haplotype on chromosome 9q33.1-q34.11. This region's targeted analysis revealed an ~1kb deletion involving in-frame loss of exon 12 of GSN predicted to result in partial deletion and fusion of two gelsolin domains of the protein. DdPCR confirmed the variant's segregation with the phenotype in extended family members (LOD score 4.52). Sanger sequencing revealed the variant to be a complex deletion-inversion-insertion event. Sequence analysis showed the variant to have likely originated from fork-stalling template-switching attributable to microhomology and formation of complex DNA structure due to multiple nested inverted and mirror repeats. Sequencing of mRNA and the amyloid protein is ongoing.

Conclusions: A complex structural variant (SV) involving exon 12 of *GSN* causes a novel form of potentially lethal 'cutaneous-internal organ amyloidosis' that is clinically, genetically and mechanistically distinct from Finnish-type amyloidosis. To our knowledge this is the first example of application of heterozygosity mapping to identify the cause of a Mendelian disorder, and of nested mirror and inverted repeats resulting in a germline SV in humans. This work highlights the value of WGS in resolving the cause of unsolved and novel disorders resulting from SVs.

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P04.008.A Clinical features and molecular characterization of three Japanese patients with autosomal dominant Robinow syndrome caused by *DVL3* variants

Kumiko Yanagi[1], *Eriko Nishi*[2], *Arisa Igarashi*[1], *Maki Omata*[1], *Yukimi Abe*[1], *Nana Kobayashi*[1], *Kazuhito Satou*[1], *Kanako Ishii*[3], *Nobuhiko Okamoto*[2], *Yoichi Matsubara*[1], *Tadashi Kaname*[1]

[1]*National Center for Child Health and Development, Setagaya, Japan,* [2]*Osaka Women's and Children's Hospital, Osaka, Japan,* [3]*Kyushu University, Fukuoka, Japan.*

Autosomal dominant Robinow syndrome 3 (DRS3, OMIM 601368) is a rare skeletal dysplasia syndrome characterized by dysmorphic features resembling a fetal face, short stature, mesomelic limb shortening, vertebral and hand anomalies. Urogenital malformations are frequently reported. The causative gene, *DVL3* (*Dishevelled 3*, NM_004423) mapped on 3q27 encodes cytoplasmic phosphoprotein DVL3 known as a homologous to Drosophila dishvelled (dsh). Dsh relays Wnt signals from receptors to downstream effectors and roles on developmental processes, including segmentation and neuroblast specification. Only eight variants have been reported to date. All are splicing or frameshift variants with premature stop codons and predicted to result in a premature termination within Dhc-C domain of DVL3. Here we report three Japanese boys who were diagnosed with DRS3 by identification of heterozygous variants in *DVL3*. Two patients (Patient 1 and patient 3) were enrolled in the IRUD (Initiative on Rare and Undiagnosed Diseases) as undiagnosed patients. One patient (Patient 2) was initially suspected of having Aarskog syndrome (OMIM 305400), but was no pathogenic variants found in the *FGD* gene by direct sequencing. Whole exome sequencing and annotated variants filtering were performed in these patients. We identified two novel frameshift variants, c.1671_1686del:p.(Tyr558Alafs*105) in Patient 1 and c.1716-1722del:p.(Ser573Alafs*93) in Patient 2, and one novel in-frame deletion, c.1861_1884del:p.(Pro621_Ala628del) in Patient 3. The in-frame deletion lacks eight amino acid residues including two phosphorylation sites, which were evolutionary conserved. The deletion would affect DVL3 polymerization leading interrupt Wnt signaling. We are presenting clinical features in each patient.

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P04.009.B Defining the molecular pathology of autosomal recessive congenital icthyosis among a cohort of Egyptian patients

Khalda S. Amr, ***Nesma Mohamad Elaraby****, Ghada Y. El-Kamah*

National Research Centre, Cairo, Egypt.

Background *Autosomal recessive congenital icthyosis* (ARCI) are heterogeneous group of rare genodermatoses characterized by marked phenotypic and genotypic variability. Non syndromic ARCI are caused by mutations in more than a dozen of distinct genes. Molecular testing for ARCI in a clinical setting is not an easy task; some genes as (*TGM1*; 190195) on chromosome 14q11.2, are relatively common while mutations in other genes are rare. **Aim**: To identify the underlying molecular pathology in a cohort of 55 non syndromic ARCI Egyptian families with at least one affected sib, using next generation sequencing (NGS).

Methods: NGS analysis was performed through a panel of 14 ARCI genes.

Results: Genetic variants could be identified in 29 (52.7%) of the 55 studied non-syndromic ARCI families. All studied patients were descending from consanguineous families and most of the characterized mutations were found in a homozygous state. Characterized variants included; sixteen pathogenic, among which are five novel missense mutations and eleven previously described variants within the *TGM1* gene. The five novel missense mutations are; (NM_000359.2) c.344T>A(Val115Asp), c.1165C>T (Arg389Cys), c.1423T>A(Cys475Ser), c.1762G>A(p.Ala588Thr), and c.1922G> (Cys641Val).

Conclusion: NGS panel analyses identified mutations within the TGM1 gene in 52.7% of studied families prioritizing it as a target gene for future molecular analyses among Egyptian ARCI patients. Limited resources prevented further whole exome analyses to help characterize the molecular pathology in the remaining almost 50% of our cohort as well as possible detection of other involved genes that might explain the marked heterogeneous phenotypes present within our patients.

K.S. Amr: None. **N.M. Elaraby:** None. **G.Y. El-Kamah:** None.

P04.011.D Bone mineralization inSATB2-associated syndrome

Anne Dittrich, ***Joyce Geelen****, Jos Draaisma*

Rradboudumc Amalia children's hospital, Nijmegen, Netherlands.

Introduction: SATB2-associated syndrome, also called Glass syndrome, is caused by pathogenic variants or deletions/duplications affecting the special AT-rich sequence binding protein 2 (*SATB2*) gene. SATB2 is a transcriptional regulatory protein involved in craniofacial and central nervous system development. Glass syndrome is characterized by intellectual disability with severely limited speech, high palate and dentofacial abnormalities. In this case series we will focus on the role of SATB2 on bone metabolism

Materials and Methods: we collected the data on bone metabolism of all children followed at the Radboudumc Amalia children's hospital.

Results: We have 4 children at follow-up with Glass syndrome. All children have signs of increased bone turnover. The oldest child had two long bone fractures at the age of 12 and low bone mineral density with a lumbar z-score of -3.5. For this reason therapy with intravenous bisphosphonate was started with good result. There were no additional fractures and the lumbar z-score increased to -1.9.

Conclusion: As shown, reduced bone mineralization in *SATB2*-associated syndrome due to high bone turnover starts at young age. In an earlier study the prevalence of decreased bone mineralization was reported to be 76% with 67% of patients had signs of increased bone turn-over. However, in a report with recommendations on the SATB2-associated syndrome, it is stated that one should consider osteopenia evaluation and optimize bone mineralization as needed. However, due to our experience, we would suggest that evaluation should be incorporated in the guideline, as preventive and therapeutic options are possible.

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P04.012.A More than meets the eye: expanding and reviewing the clinical and mutational spectrum of brittle cornea syndrome

*Tibbe Dhooge[1], **Tim Van Damme**[1], Delfien Syx[1], Laura Muiño Mosquera[1,2], Sheela Nampoothiri[3], Anil Radhakrishnan[4], Pelin Ozlem Simsek-Kiper[5], Gülen Eda Utine[5], Maryse Bonduelle[6], Isabelle Migeotte[7], Osama Essawi[1], Serdar Ceylaner[8], Adila Al Kindy[9], Brad Tinkle[10], Sofie Symoens[1], Fransiska Malfait[1]*

[1]Center for Medical Genetics, Department of Biomolecular Medicine, Ghent University and Ghent University Hospital, Ghent, Belgium, [2]Divison of Pediatric Cardiology, Department of Pediatrics, Ghent University Hospital, Ghent, Belgium, [3]Department of Pediatric Genetics, Amrita Institute of Medical Sciences & Research Centre, Cochin, India, [4]Department of Ophthalmology, Amrita Institute of Medical Sciences & Research Centre, Cochin, India, [5]Department of Pediatric Genetics, Faculty of Medicine, Hacettepe University, Ankara, Turkey, [6]Centre for Medical Genetics, Universitair Ziekenhuis Brussel, Brussels, Belgium, [7]Center of Human Genetics, Université Libre de Bruxelles, Brussels, Belgium, [8]Intergen Genetic Research Center, Ankara, Turkey, [9]Department of Genetics, College of Medicine, Sultan Qaboos University, Muscat, Oman, [10]Division of Medical Genetics, Peyton Manning Children's Hospital, Indianapolis, IN, USA.

Brittle cornea syndrome (BCS) is a rare autosomal recessive disorder characterized by corneal thinning and fragility, leading to corneal rupture, the main hallmark of this disorder. Non-ocular symptoms include hearing loss, but also signs of connective tissue fragility, placing it in the Ehlers-Danlos syndrome (EDS) spectrum. It is caused by biallelic pathogenic variants in *ZNF469* or *PRDM5*, which presumably encode transcription factors for extracellular matrix components. We report the clinical and molecular features of nine novel BCS families, four of which harbor variants in *ZNF469* and five in *PRDM5*. We also performed a genotype and phenotype-oriented literature overview of all (N = 85) reported patients with *ZNF469* (N = 53) and *PRDM5* (N = 32) variants. Musculoskeletal findings may be the mean reason for referral, and often raise suspicion of another heritable connective tissue disorder such as kyphoscoliotic EDS, osteogenesis imperfecta or Marfan syndrome, especially when corneal rupture has not yet occurred. Our findings highlight the multisystemic nature of BCS and validate its inclusion in the EDS classification. Importantly, gene panels for heritable connective tissue disorders should include *ZNF469* and *PRDM5* to allow for timely diagnosis and appropriate preventive measures for this rare condition.

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P04.013.B Bilateral and symmetrical enchondromatosis lesions: clinical, radiologic and genetic findings

***Pauline Marzin**[1,2], Celine Huber[2], Genevieve Baujat[1,2], Caroline Michot[1,2], Agnès Guichet[3,4], Estelle Colin[3,4], Michel Panuel[5], Valérie Cormier-Daire[1,2]*

[1]Service de Génétique clinique et Centre de référence pour les maladies osseuses constitutionnelles, AP-HP, Hôpital Necker-Enfants Malades, 75015 Paris, France, [2]Université de Paris, Laboratory of Molecular and Physiopathological Bases of Osteochondrodysplasia, INSERM UMR 1163, Imagine Institute, 75015 Paris, France, [3]Biochemistry and Genetics Department, University Hospital of Angers, Angers, France, [4]MitoLab, UMR CNRS 6015-INSERM, MitoVasc Institute, University of Angers, Angers, France, [5]Aix Marseille University, CNRS, EFS, ADES, Marseille, France.

Enchondromas are benign cartilaginous tumors, typically localized in the metaphyses. The most common enchondromatosis, Ollier disease and Maffucci syndrome related to *IDH1* and *IDH2*, are characterized by asymmetrical lesions. Bilateral and/or symmetrical enchondromas can be seen in rarer enchondromatosis such as metachondromatosis, dyspondyloenchondromatosis, genochondromatosis and spondyloenchondrodysplasia (SPENCD, related to recessive variants in *ACP5*). Even though variants in *PTPN11* and *COL2A1* have been identified in metachondromatosis and dyspondyloenchondromatosis, respectively, molecular basis of bilateral and/or symmetrical enchondromas remain unknown. We performed whole exome sequencing analysis from blood samples in a cohort of 11 individuals from eight families presenting with bilateral and symmetrical enchondromas. We identified variants in known genes in three families of the eight (38%), including a mosaic variant in the *IDH1* gene in two patients, with symmetrical enchondromas and a large homozygous deletion encompassing *ACP5* in one patient with SPENCD. Variants of uncertain significant (VOUS) were identified in two other families, including a heterozygous nonsense variant in the *PELI2* gene, encoding an interleukin-1 receptor-associated kinase (IRAK)-interacting proteins, in three affected members from the same family and a *de novo* heterozygous 1.5 Mb deletion of 12p12.1p11.22 in a patient with enchondroma and type E brachydactyly. No candidate variant was identified in three families including one family with recurrent sibs. These findings support the need of increasing the number of families tested and/or the analysis of affected tissues.

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P04.014.C Sitting-to-standing height ratio is a sex-specific risk factor for chronic back pain

***Maxim B. Freidin**[1], Yakov A. Tsepilov[2,3], Yurii S. Aulchenko[2,3], Pradeep Suri[4], Frances M. K. Williams[1]*

[1]King's College London, London, United Kingdom, [2]Novosibirsk State University, Novosibirsk, Russian Federation, [3]PolyOmica, 's-Hertogenbosch, Netherlands, [4]University of Washington, Seattle, WA, USA.

Background: Chronic back pain (CBP) is more common among females for reasons not fully understood. We hypothesized that sitting-to-standing height ratio, known to be different between the sexes, may contribute.

Methods: We used European individuals from UK Biobank (project #18219) comprising 222,361 males and 263,602 females to test this hypothesis. Logistic regression was used to assess the association of CBP with sitting height, standing height, and their ratio while adjusting for age, BMI, and job involving prolonged standing and heavy lifting. Mediation analysis and Mendelian randomization (MR) were applied to assess causality. Instruments for MR were selected from publicly available GWAS data for sitting-to-standing height ratio, ensuring non-overlap with chronic BP GWAS using UK Biobank.

Results: Both sitting and standing height exhibited a weak association of similar magnitude for CBP in both sexes (Table). However, sitting-to-standing height ratio was associated with greater odd of CBP in males (Table) but with lower odds in females (Table). Mediation analysis showed both direct and BMI-mediated risk effects of the ratio on CBP in males; while in females there was BMI-mediated risk effect and direct protective effect. MR supported a causal impact of sitting-to-standing height ratio on CBP risk in females, but not in males.

Conclusion: We provide evidence that different body proportions seen in men and women may contribute to the different risk of CBP between the sexes.

Odds ratios for anthropometric traits over chronic back pain

Trait	Males	Females
Standing height	1.013 (1.011-1.016)	1.011 (1.009-1.014)
Sitting height	1.030 (1.026-1.034)	1.013 (1.008-1.017)
Sitting-to-standing height ratio	14.373 (4.819-42.867)	0.139 (0.050-0.382)

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P04.016.A Transcriptomic diagnosis of a deep intronic CLCN7 mutation

Odelia Chorin[1], *Naomi Yachelevich*[2], *Khaled Mohamed*[3], *Ilana Moscatelli*[4], *John Pappas*[1], *Kim Henriksen*[3], *Gilad D. Evrony*[1,5]

[1]*Center for Human Genetics and Genomics, New York, NY, USA,* [2]*Division of Clincal Genetic Services, Department of Pediatrics, New York, NY, USA,* [3]*Nordic Biosciences Biomarkers and Research, Harlev, Denmark,* [4]*Division of Molecular Medicine and Gene Therapy, Lund University, Lund, Sweden,* [5]*Department of Pediatrics, and Department of Neuroscience & Physiology, New York University Grossman School of Medicine, New York, NY, USA.*

Background Over half of children with rare genetic diseases remain undiagnosed despite maximal clinical evaluation and DNA-based genetic testing. As part of an Undiagnosed Diseases Program applying transcriptome (RNA) sequencing to identify the causes of these unsolved cases, we studied a child with severe infantile osteopetrosis leading to cranial nerve palsies, bone deformities, and bone marrow failure, for whom whole-genome sequencing was nondiagnostic.

Methods: We performed transcriptome (RNA) sequencing of whole blood followed by analysis of aberrant transcript isoforms and osteoclast functional studies.

Results: We identified a pathogenic deep intronic variant in *CLCN7* creating an unexpected, frameshifting pseudoexon causing complete loss of function. Functional studies, including osteoclastogenesis and bone resorption assays, confirmed normal osteoclast differentiation but loss of osteoclast function.

Conclusion: This is the first report of a pathogenic deep intronic variant in *CLCN7*, and our approach provides a model for systematic identification of noncoding variants causing osteopetrosis—a disease for which molecular-genetic diagnosis can be pivotal for potentially curative hematopoietic stem cell transplantation. Our work illustrates that cryptic splice variants may elude DNA-only sequencing and supports broad first-line use of transcriptome sequencing for children with undiagnosed diseases.

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P04.017.B Two novel variants in *MMP13* gene in a Czech family with metaphyseal anadysplasia type 1

Lucie Hrušková[1], *Helena Paszeková*[1], *Ivo Mařík*[2,3], *Veronika Krulišová*[1], *Daniela Zemková*[4], *Anna Vážná*[5], *Zděnka Vlčková*[1], *Renata Michalovská*[1]

[1]*GHC Genetics, s.r.o., Prague, Czech Republic,* [2]*Ambulant Centre for Defects of Locomotor Apparatus, Prague, Czech Republic,* [3]*Faculty of Health Care Studies, West Bohemia University, Pilsen, Czech Republic,* [4]*Motol University Hospital, Prague, Czech Republic,* [5]*Department of Anthropology and Human Genetics, Faculty of Science, Charles University, Prague, Czech Republic.*

Introduction: Metaphyseal anadysplasia type 1 is an autosomal dominant skeletal disease characterized by metaphyseal changes, epiphyseal dysplasia and rhizomelic shortening of limbs followed by regression of symptoms with later growth. Genetic origin is associated with pathogenic variants in *MMP13* gene. Matrix metalloproteinase 13 encoded by this gene plays important roles in bone formation and growth.

Materials and Methods: We performed massive parallel sequencing of gDNA isolated from whole blood on NextSeq (Illumina) using panel Clinical Exome (CES) kit by Sophia Genetics, followed by Sanger sequencing and CNV analysis. This solution consists of 116,355 individually designed probes that span approx. 11 Mb of target regions covering more than 4,900 genes, with known mendelian/inherited disease-causing mutations.

Results: Molecular genetic analysis identified two previously unreported heterozygous cis-variants c.236A>C and c.251T>G in *MMP13* in a Czech boy suspected with metaphyseal anadysplasia, type 1. Clinical-radiological examination showed rhizomelic shortness of stature, femoral bowing, abnormality of metaphyses, delayed ossification of carpal bones and patella. The same bone dysplasia occurs in the sister, mother, maternal aunt and maternal grandfather. Subsequent molecular genetic testing in these family members showed that the two variants described above in the *MMP13* gene also occur in them and thus segregate with the occurrence of the disease in the family.

Conclusion: Our findings demonstrate the efficiency of exome sequencing approach in determination of molecular background in differential diagnosis not only of skeletal dysplasias. Further, our findings expand genotype spectrum of *MMP13* associated disorders and offer precise diagnosis of metaphyseal anadysplasia type 1.

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P04.018.C Very early diagnosis of Cole-Carpenter syndrome: novel variant and maternal mosaicism

Daniele Guadagnolo[1], *Gioia Mastromoro*[1], *Enrica Marchionni*[1], *Francesca Di Palma*[1], *Claudia Cesario*[2], *Mario Roggini*[3], *Antonio Novelli*[2], *Antonio Pizzuti*[1]

[1]*Department of Experimental Medicine, Sapienza University of Rome, Rome, Italy,* [2]*Laboratory of Medical Genetics, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy,* [3]*Department of Pediatrics, Sapienza University of Rome, Rome, Italy.*

Introduction: Fetal abnormal head shape represents a significant challenge in prenatal diagnosis. It can be the initial presentation of several disorders, requiring multidisciplinary approaches.

Materials and methods: A 35-year-old woman was referred at 34 gestational weeks for unusual fetal head shape. Chromosomal molecular analysis, performed after amniocentesis for multiple soft markers, was normal. After caesarean delivery at 38 gestational weeks, the baby was in normal length and weight ranges, but macrocephaly, micrognathia and broad nasal bridge were noted. Total body X-rays and *trio* Clinical Exome Sequencing (CES) were requested.

Results: X-rays showed dolicocephaly, ossified metopic suture, widening of the bregmatic fontanella and of the sagittal and

lambdoideal sutures, perisutural calcified spots. CES detected a novel heterozygous missense variant in *P4HB* (prolyl 4-hydroxylase subunit b; MIM*176790) in the proband (NM_000918.4:c.1375G>A; NP_000909.2:p.Gly459Arg;rs749458033). The same variant was found in heterozygosis with 34% mosaicism rate in the mother. Heterozygous *P4HB* mutations cause Cole-Carpenter syndrome 1 (MIM#112240). The variant was not described in literature, HGMD, ClinVar or DECIPHER. It is very rare (GnomADv2.1.1: 0.000016) and has high predicted deleteriousness. It is classified as Likely Pathogenic. Cole-Carpenter syndrome is characterized by dysmorphisms, wide sutures and fontanelles, short stature, proptosis, hydrocephalus, bone fragility, no intellectual disability. Somatic mosaicism for a pathogenic *P4HB* has already been described in the healthy father of an affected child.

Conclusion: Early molecular analysis for skull anomalies can guide subsequent clinical approaches but also yield complex results. We suspect that parental mosaicism might be more common than previously thought for *P4HB* variants.

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P04.020.A hiPSC-derived epidermal keratinocytes from ichthyosis patients show altered expression of cornification markers

*Dulce Lima Cunha[1,2,3], Amanda Oram[1], Robert Gruber[4], Roswitha Plank[1,2], Arno Lingenhel[2], Manoj Kumar Gupta[5], Janine Altmüller[6], Peter Nürnberg[6], Mariya Moosajee[3], Matthias Schmuth[4], Johannes Zschocke[2], Tomo Šarić[5], Katja Martina Eckl[2,7], **Hans Christian Hennies**[1,2,6]*

[1]Department of Biological and Geographical Sciences, University of Huddersfield, Huddersfield, United Kingdom, [2]Institute of Human Genetics, Medical University of Innsbruck, Innsbruck, Austria, [3]Institute of Ophthalmology, UCL, London, United Kingdom, [4]Department of Dermatology, Medical University of Innsbruck, Innsbruck, Austria, [5]Center for Physiology and Pathophysiology, Institute for Neurophysiology, University Hospital Cologne, Cologne, Germany, [6]Cologne Center for Genomics, University Hospital Cologne, Cologne, Germany, [7]Department of Biology, Edge Hill University, Ormskirk, United Kingdom.

Introduction: Inherited ichthyoses represent a heterogeneous group of skin disorders characterised by impaired epidermal barrier function and disturbed cornification. Current knowledge about disease mechanisms has been uncovered mainly through mouse models or human organotypic models. However, most mouse lines suffer from severe epidermal barrier defects causing neonatal death and human keratinocytes have very limited proliferation ability in vitro.

Results: We have generated human induced pluripotent stem cells (hiPSCs) from patients with congenital ichthyosis, either non-syndromic autosomal recessive congenital ichthyosis (ARCI) or the ichthyosis syndrome trichothiodystrophy (TTD). hiPSCs were successfully differentiated into basal keratinocytes (hiPSC-bKs), with high expression of epidermal keratins 5 and 14. Terminal differentiation of hiPSC-bKs was induced and markers keratin 1 and involucirn were expressed. TTD1 hiPSC-bKs showed reduced expression of *FLG* and *SPRR2B* in line with previous reports and mouse models. ARCI hiPSC-bKs showed more severe defects, with downregulation of several genes involved in epidermal ceramide metabolism. Differences to findings in ARCI patients might point to the importance of specific mutations and an influence of patient treatment on their keratinocyte expression profiles.

Conclusions: Our results demonstrate the successful generation of hiPSC-based in vitro models mimicking the disease phenotypes, proving a valuable system for molecular investigations and drug development. Studies with organotypic models will be used to further characterize functional changes associated with ichthyosis. Patient-derived hiPSCs can be a long-term source for a variety of different cells and significantly contribute to the development of complex skin disease models, ultimately facilitating the translation of therapeutic approaches into clinical studies.

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P04.021.B Four novel *EFNB1* variants found through sequencing-based methods in female patients with craniofrontonasal syndrome

***Ewelina Bukowska-Olech**[1], Anna Jakubiuk-Tomaszuk[2], Maria Jedrzejowska[3], Ewa Obersztyn[4], Pawel Gawlinski[4], Michal Piechota[5], Marta Bielska[6], Aleksander Jamsheer[1,5]*

[1]Department of Medical Genetics, Poznan University of Medical Sciences, Poznan, Poland, [2]Department of Pediatric Neurology and Rehabilitation, Medical University of Bialystok, Bialystok, Poland, [3]Department of Medical Genetics, The Children's Memorial Health Institute, Warsaw, Poland, [4]Department of Medical Genetics, Institute of Mother and Child, Warsaw, Poland, [5]Centers for Medical Genetics GENESIS, Poznan, Poland, [6]Department of Pediatrics, Hematology, Oncology and Diabetology, Medical University of Lodz, Lodz, Poland.

Background: Craniofrontonasal syndrome (CFNS) is a rare X-linked disorder that results from pathogenic variants in the *EFNB1* gene. The syndrome inherits in a paradoxical manner and exceptionally presents greater severity of symptoms in heterozygous females than hemizygous males.

Materials and methods: We recruited five sporadic (patients 1-4) and one familial case (patients 5& 6), all of whom presented a spectrum of craniofacial abnormalities. Of note, we reported discordant phenotype in twin female patients 5& 6. We applied either targeted next-generation sequencing (NGS) of a custom gene panel or PCR and Sanger sequencing to achieve a molecular diagnosis. Besides, we run both zygosity analysis and X chromosome inactivation (XCI) assay for twin patients 5& 6.

Results: First, we reported three additional novel variants in the *EFNB1* gene: p.(Trp12*), p.(Tyr73Met*fs*86), p.(Glu210*), and one known: p.(Cys64Phe). Second, we confirmed the monozygosity of patients 5& 6. Finally, we showed random XCI in twin patient 5 (46% vs 54%), who presents milder phenotype in comparison to her twin sister (patient 6) having non-random XCI (84% vs 16%).

Conclusion: Our findings provide valuable molecular data that may be applied both in diagnostics and genetic counselling. We have described the intriguing differences of the clinical phenotype in the monozygotic twin patients 5& 6 harbouring an identical p.(Glu210*) variant and we have pointed to the presence of unusual phenomenon such as mildly affected females with CFNS.

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P04.022.C Comprehensive genetic screening in patients with syndromic craniosynostosis

Trayan N. Delchev, *Savina Hadjidekova, Hadil Kathom, Stoyan Bichev, Tsvetina Veleva, Iliana Boneva, Daniela Avdjieva-Tzavella*

Medical University-Sofia, Sofia, Bulgaria.

Introduction: Syndromic craniosynostosis is a genetically determined premature ossification and closure of one or more of the cranial sutures. This may result in severe dysmorphism, increased intracranial pressure, seizures, visual and hearing defects, psychomotor delay and behavioural anomalies. Syndromic craniosynostosis is also commonly associated with additional malformations and dysmorphic features.

Materials and Methods: We present our comprehensive genetic investigation of 39 patients with syndromic craniosynostosis screened systematically with a combination of cytogenetic analysis, multiplex ligation-dependent probe amplification (MLPA) and array-based comparative genomic hybridisation (aCGH).

Results: Pathological findings were established in 15.3% (6/39) of the cases using aCGH, 8.33% (3/39) using MLPA and 2.85% (1/39) using conventional karyotyping. About 12.8% (5/39) of the patients with normal karyotype carried submicroscopic chromosomal rearrangements.

Conclusions: In our study array-based comparative genomic hybridisation had the highest detection rate compared to chromosome analysis and MLPA. „Gain of function" variations and duplications were the predominant type of genetic defect we found. This suggests the leading role of those findings in the pathogenesis of syndromic craniosynostosis.

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P04.023.D Analysis of novel splice site variants in craniosynostosis causing genes

Angela Borst[1], *Tillmann Schweitzer*[2], *Denise Horn*[3], *Erdmute Kunstmann*[1], *Eva Klopocki*[1]

[1]*Institute of Human Genetics University of Wuerzburg, Würzburg, Germany,* [2]*Department of Pediatric Neurosurgery University Hospital of Wuerzburg, Würzburg, Germany,* [3]*Institute for Medical and Human Genetics Charité Universitätsmedizin Berlin, Berlin, Germany.*

Congenital malformation of the skull are rare disease conditions, which may have severe impact on the life of patients. Craniosynostosis appears with a prevalence of 1 in 2500 newborns and represents a premature fusion of one or more cranial sutures. The disease can either occur as part of a syndrome or as non-syndromic, isolated craniosynostosis. The Craniosynostosis 3 (MIM 615314) phenotype, a molecular well-described form of isolated craniosynostosis, is caused by mutations in the *TCF12* gene. In contrast to *TCF12*, mutations in the *FGFR2* gene are multifaceted and are associated with diverse syndromes, including craniosynostosis syndromes, i.e. Apert (MIM 101200), Pfeiffer (MIM 101600) and Saethre-Chotzen (MIM 101400) syndromes.

The aim of our study was, to identify novel genetic variants in craniosynostosis associated genes by Next Generation Sequencing (NGS) using a specific craniosynostosis gene panel and to further analyse the detected genetic variants. In a proportion of cases changes in the splicing process of the corresponding gene were predicted in silico. To validate the consequences of the splice site variants on correct transcript splicing, we performed in vitro splice assays with mutation-matched minigene constructs in U2OS cells. In total, we investigated four potential splice site variants in *TCF12* and *FGFR2*. In three patients with coronal synostosis, the variants lead to an aberrant splicing (exon skipping, inclusion of intronic sequence) of the *TCF12* transcript. Taken together, we could validate novel splice site mutations in the *TCF12* and *FGFR2* genes and extend the mutational spectrum.

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P04.024.A TRAF3 and NBR1 both influence the effect of CYLD (Arg936X) mutation on NF-κB activity

Judit Danis[1,2,3], *Evelyn Kelemen*[3,4], *Neil Rajan*[5], *Nikoletta Nagy*[1,3], *Márta Széll*[1,3], *Éva Ádám*[3]

[1]*ELKH-SZTE Dermatological Research Group, Szeged, Hungary,* [2]*HCEMM-USZ Dermatological Research Group, Szeged, Hungary,* [3]*Department of Medical Genetics, University of Szeged, Szeged, Hungary,* [4]*Department of Dermatology and Allergology, University of Szeged, Szeged, Hungary,* [5]*Institute of Genetic Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom.*

Recently the cases of a Hungarian and an Anglo-Saxon pedigrees has been presented, who are affected by CYLD cutaneous syndrome (syn: Brooke-Spiegler syndrome), carry the same disease-causing mutation (c.2806C>T, p.Arg936X) of the cylindromatosis *(CYLD)* gene but exhibit striking differences in their phenotypes. By whole exome sequencing, missense genetic variants of the *TRAF3* and *NBR1* genes were identified from affected family members of the Hungarian family, that are not present in the Anglo-Saxon family, suggesting their affected proteins (TRAF3 and NBR1) are putative phenotype-modifying factors. To clarify how wild type and mutant TRAF3 and NBR1 modify the effect of CYLD on NF-κB signal transduction pathway, an in vitro experimental system was set up. Our study revealed that the combined expression of mutant CYLD(Arg936X) both with TRAF3 and NBR1 caused increased NF-κB activity, regardless of the latter two proteins being wild type or mutant. We conclude that increased expression levels of these proteins further strengthen the effect of CYLD(Arg936X) mutation on the NF-κB activity in HEK293 cells and may explain the phenotype modifying effect of these genes in CYLD cutaneous syndrome.

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P04.025.B Dedifferentiated liposarcoma: Case report

Fiorita G. L. Mundim[1,2], *Wesley R. S. Liberato*[1], *Reigson A. Dias*[1], *Leticia Benevenutti*[1], *Felipe R. C. Meira*[1], *Katia M. T. C. Castro*[1], *Pedro G. L. Mundim*[1], *Nathalia T. Sousa*[1], *Renan Reis*[2]

[1]*UNIFENAS, Alfenas/MG, Brazil,* [2]*UNIVAS, Pouso Alegre/MG, Brazil.*

Introduction: Dedifferentiated liposarcoma (DDL) is considered a well-differentiated form of histological (tumor) progression of liposarcoma. Around 15% of cases have the potential to metastasize and with a high rate of recurrence. Since DDL can morphologically mimic other sarcomas that have higher rates of metastasis, especially in the retroperitoneum and inguinal canal, the diagnosis and correct classification of DDL are essential to determine an adequate prognosis and treatment for the patient.

Materials and Methods: In the present case report, it is a female patient, 39 years old, residing in Pouso Alegre, Brazil, in October 2019. She started with pain in the left gluteal region associated with nodular lesion in the place with progressive

growth. The biopsy showed a mesenchymal neoplasia. Subsequent immunohistochemical examination revealed a tumor compatible with a Dedifferentiated liposarcoma.

Results: DDL is a cellular and typically non-lipogenic sarcoma with significant pleomorphism. Molecularly characterized by a ring or a giant marker / rod chromosomes composed of material from 12q13-15. Most DDL also have gene amplification MDM2 and CDK4, which may be similar to other tumors (Intimal sarcoma, Parosteal osteosarcoma, low grade central osteosarcoma, etc.)

Conclusion: For the diagnosis and correct classification of the DDL, it is essential to carry out a cytogenetic study, Immunostaining for MDM2 and CDK4 or molecular testing for 12q13-15 amplification.

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P04.027.D Targeted next generation sequencing of Hypohidrotic ectodermal dysplasia in Egyptian pedigrees

Hoda Abdalla Ahmed Radwan[1], *Khalda Sayed Amr*[1], *Eman Rabie*[1], *Ghada El-Kamah*[1], *Inas Sayed*[1], *Mennat Mehrez*[1], *Nehal Hassib*[1], *Suher Zada*[2], *Maha Abuzaid*[1], *Mohamed Abdel-Kader*[1], *Mostafa Mostafa*[1], *Yasmine Mohsen*[1]

[1]*National Research Centre, Cairo, Egypt,* [2]*American University, Cairo, Egypt.*

Introduction: Ectodermal dysplasia (ED) defines a group of genetic disorders characterized by developmental defects of 2 or more structures of ectodermal origin. Pathogenesis is governed by defects in many genes involved in multiple developmental pathways. Hypohidrotic ectodermal dysplasia (HED) is the most common ED form characterized by hypodontia, hypohidrosis, hypotrichosis and in several instances dysmorphic features. Mutations in four genes: EDA, EDAR, EDARADD and WNT10A were reported to contribute to 90% of HED cases. The aim of our study is to assess the clinical and molecular profiles of HED in Egyptian patients.

Patients and Methods: Thirty-five HED patients descending from 32 unrelated pedigrees were clinically diagnosed based on thorough clinical and dental examination. Patients were tested using an NGS custom panel comprising the four aforementioned genes. Sanger sequencing was performed for variant confirmation and segregation analysis. Pathogenicity of variants was assessed according to American College of Medical Genetics (ACMG) guidelines as well as bioinformatics tools (e.g., Polyphen, SIFT, and Alamut).

Results: We identified 12 pathogenic mutations: six novel and six previously reported mutations. A molecular basis was identified in 16/32 pedigrees. EDA was the most frequent (13/16), followed by EDARADD (2/16) and EDAR (1/16). No variants were identified in the WNT10A gene.

Conclusion: The EDA, EDAR and EDARADD harbored the molecular pathology in 50% of the studied HED patients. That might be attributed to different ethnic backgrounds, and further analyses through exome sequencing of the uncharacterized patients might confirm rare genes involvement or identify new genes governing ED pathogenesis.

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P04.028.A Peds2Gene study: clinical and molecular delineation of a Spanish cohort of pediatric patients with Ehlers-Danlos Syndrome

Laura Plaza, *Antonio Federico Martinez-Monseny, Dídac Casas, Asunción Vicente, Francesc Palau*

Hospital Sant Joan de Deu, Barcelona, Spain.

Introduction: Ehlers-Danlos Syndrome (EDS) is a group of hereditary connective tissue disorders that manifests as skin hyperextensibility, hypermobility of joints and fragility of blood vessels. The hypermobility-type EDS (hEDS) is the most frequent and its molecular cause is unknown. Diagnosing EDS is challenging due to the high clinical variability and the lack of a diagnostic biomarker in paediatrics.

Materials and Methods: Prospective and retrospective observational study at a tertiary pediatric hospital including patients < 18 years at the date of diagnosis with EDS between 2012-2020. The data was collected reviewing clinical records.

Results: Thirty-five patients were included; hEDS (65.7%), classical (22.8%), vascular (5.7%), kyphoscoliotic (2,8%) and dermatosparaxis types (2.8%). Most of them were women (62.9%). Musculoskeletal symptoms were the commonest complication (91.4%), with chronic pain of joints being the most frequent feature (62.9%). Psychiatric disorders were present in 54.3% of patients, with the most frequent being anxiety and depression (22.8%). A higher Beighton score did not correlate with musculoskeletal, dermatologic, or other complications. Atrophic scarring and easy bruising were less frequent in hEDs patients ($p < 0.000$,$p < 0.002$). Chronic abdominal pain was significantly associated with migraine ($p < 0.002$). All the types have molecular confirmation except the hEDS, but we found some genes (TNXB,ELN,PIEZO2) that could be implicated, requiring future studies.

Conclusions: This is the first Spanish study focusing on pediatric EDS. We propose interesting and novel genotype-phenotype aspects. We provide detailed insights into the potential complications during childhood to help minimize the physical and emotional impact of the syndrome in patients and their families.

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P04.029.B New variant in TANGO1 gene in a patient with hypermobile type of Ehlers-Danlos syndrome

Anna Junkiert-Czarnecka, *Maria Pilarska-Deltow, Aneta Bąk, Marta Heise, Olga Haus*

Department of Clinical Genetics Collegium Medicum in Bydgoszcz, Bydgoszcz, Poland.

Introduction: Hypermobile Ehlers-Danlos syndrome (hEDS) is a non-inflammatory connective tissue disorder. It is perhaps the most common hereditary connective tissue disorder. hEDS unlike other types of EDS has no known genetic etiology. One of the dysfunctions in hEDS patients' cells is the impaired transport of extracellular matrix proteins from cells to intercellular space. A protein taking part in this process is TANGO1 encoded by the *TANGO1* gene. The aim of the study was a sequencing analysis of *TANGO1* gene and an attempt to evaluate its potential role in the etiology of the hypermobile type of Ehlers-Danlos syndrome.

Methods: The study was carried out on a patient with a hypermobile type of EDS. It was a 48-year-old woman presented with: joints hypermobility, recurrent dislocations, whole body pain, easy bruising, striae, gothic palate and arachnodactyly. Her daughter had mild symptoms of hEDS. Sequencing analysis of *TANGO1* was performed using the Sanger sequencing technique. For bioinformatic analysis, VarSome tool was applied.

Results: In studied patient variant c.5637_5638insA (p. Leu1880ThrfsTer6) in *TANGO1* gene was detected. This variant was not described up to now in EDS patients, and according to VarSome it is pathogenic.

Conclusion: Analysis of the role of the *TANGO1* gene in the etiology of hypermobile type of EDS is a new approach to evaluating the genetic background of this disorder. The impact of a pathogenic variant in *TANGO1* on its function and resulting extracellular matrix condition need further investigations. This investigation was financed by a grant from National Science Centre Poland (2018/29/N/NZ5/00345).

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P04.030.C Molecular spectrum in 23 Spanish families affected by hereditary multiple osteochondromas

M C. Martínez-Romero[1,2,3], *A T. Serrano-Antón*[1,2,4], *M J. Sánchez-Soler*[1,2,3], *L Rodríguez-Peña*[1], *M J. Ballesta-Martínez*[1,2,3], *V López-González*[1,2,4], *M Barreda-Sánchez*[1,3], *M E. Pérez-Tomás*[1], *P Carbonell-Meseguer*[5,2], *C Salcedo-Cánovas*[6], *E Guillén-Navarro*[1,2,4]

[1]*Sección de Genética Molecular (Centro de Bioquímica y Genética Clínica), Sección de Genética Médica (Servicio de Pediatría), Hospital Clínico Universitario Virgen de la Arrixaca. Instituto Murciano de Investigación Biosanitaria (IMIB-Arrixaca), El Palmar (MURCIA), Spain,* [2]*CIBERER, Centro de Investigación Biomédica en Red de Enfermedades Raras, ISCIII, Madrid, Spain,* [3]*Cátedra de Genética Médica. UCAM-Universidad Católica de Murcia, Murcia, Spain,* [4]*Departamento de Pediatría. Facultad de Medicina. UMU-Universidad de Murcia, Murcia, Spain,* [5]*Sección de Genética Molecular (Centro de Bioquímica y Genética Clínica), Sección de Genética Médica (Servicio de Pediatría). Hospital Clínico Universitario Virgen de la Arrixaca. Instituto Murciano de Investigación Biosanitaria (IMIB-Arrixaca), El Palmar (MURCIA), Spain,* [6]*Servicio de Traumatología. CSUR ortopedia. Hospital Clínico Universitario Virgen de la Arrixaca, El Palmar (MURCIA), Spain.*

Introduction: Osteochondroma represents the most common benign osseous tumour, 20-50%; in familiar occurrence two AD diseases are probable: hereditary multiple osteochondromas (HMO)(ORPHA:321) associated with variants in EXT1(MIM,#133700) or EXT2(MIM,#133701) genes and metachondromatosis (MC)(ORPHA:2499), caused in PTPN11(MIM,#156250). Both disorders have risk of malignant transformation (3-5%). Our goal is to find out the molecular spectrum in a cohort of Spanish patients with multiple osteochondroma.

Methodology: Coding and flanking exons of EXT1 (NM_000127.2), EXT2(NM_207122.) and PTPN11(NM_002834.4) genes were performed by capture (SureSelectQXT®Agilent) and sequenced by Illumina platform. Minimum depth coverage was 20x. Copy Number Variation (CNV) were evaluated using DecoN algorism and MLPA-P215-B3-EXT-Kit.

Results: 23 index patients (12 females/11 males) with average age of 15 years were included. Distribution of variants by genes were EXT1(56.5%), EXT2(34.8%) and PTPN11(8.7%). *De novo* variants represented 56.5% cases *versus* 44.5% inherited. A total of 22 pathogenic variants (15 novel/7 reported in HGMD®2020.4) were identified, most of them privates (22/23) being only recurrent one variant in two cases (EXT1:c.936-11insTG). Type of variants: frameshift (45.4%), nonsense (27.3%), splicing (13.6%), CNV (9%) and missense (4.5%). Anatomical distribution referred of osteochondroma was mainly in long-bone extremities (65.2%) and hands (43.5%), nevertheless genotype-phenotype correlation by genes was not observed; in 7 patients (30.4%) osseous deformities required surgery but malignant degeneration was not notified.

Conclusion: -There is a broad allelic heterogeneity in EXT1 and EXT2 genes resulting with 15 novel variants identified. -Secondary variants in PTPN11 gene highlights the importance of expanding the study to other HMO genes for a more suitable personalized treatment.

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P04.031.D Father and daughter with acromicric dysplasia

Cinzia Mautone[1], *Mario Francesco Iasevoli*[1], *Pamela Paglia*[1], *Giuseppina Barra*[1], *Dario Di Salvio*[2], *Mariateresa Falco*[3], *Carolina Mauro*[3], *Emanuele Agolini*[4], *Antonio Novelli*[4], *Daniela Melis*[1,3]

[1]*Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana" University of Salerno, Baronissi (SA), Italy,* [2]*Department of Maternal-Infant, Pediatric Section, University of Naples Federico II, Napoli, Italy,* [3]*Pediatric Unit San Giovanni di Dio e Ruggi d'Aragona University Hospital, Salerno, Italy,* [4]*Departmnent of Laboratories, Medical Genetic Unit Bambino Gesù Children's Hospital, Roma, Italy.*

Introduction: Mutations of FBN1 are responsible for different disorders including Acromicric dysplasia (characterized by short hands and feet, joint limitations), Geleophysic dysplasia (progressive heart involvement), and Weill-Marchesani syndrome (microspherophakia).Case report: We describe father and daughter with short stature with no other systemic involvement. Father was evaluated from first years of life for congenital hip dysplasia and short stature; he underwent GH therapy with poor results. His childhood X-rays showed shortness of long bones and hands' phalanges and typical notches of II and V metacarpus. At physical examination both showed round face, well-defined eyebrows, bulbous nose with anteverted nostrils, disharmonic short stature with short limbs, relative macrocephaly, and brachydactyly; hand camptodactily was detected during childhood in the father and in the child at 4 years of age. No joint stiffness was recored during adulthood in the father. NGS panel of genes involved in skeletal dysplasias found an heterozygous variant (c.5285G>A) in FBN1 gene in both father and daughter, previously unreported; a different pathogenetic variant involving Glycine 1762 has been associated with both Acromicric and Geleophysical dysplasia. No cardiac or ocular involvement were present, thus a diagnosis of Acromicric dysplasia was proposed.

Conclusion: We report a familial case of Acromicric dysplasia, with a novel variant in FBN1 gene. Clinical features of both father and daughter overlap. The father underwent GH therapy in childhood, with poor results. The report could expand clinical features of this condition and help to define natural history of the disease.

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P04.032.A Aarskog-Scott syndrome: case report and updated-review

Marta Spodenkiewicz[1], *Gauthier Loron*[2,3,4], *Céline Poirsier*[1], *Francesco Laconi*[5], *Marie Massier*[1], *Perrine Venot*[2], *Clémence Jacquin*[6], *Lucas Hérissant*[1], *Emilie Landais*[1], *Martine Doco-Fenzy*[1,7,8]

[1]*Service de génétique, CHU de Reims, Reims, France,* [2]*Service Pédiatrie B, CHU Reims, Reims, France,* [3]*Faculté de médecine, Reims, France,* [4]*CReSTIC/ EA3804, Reims, France,* [5]*Service de chirurgie*

Pédiatrique, CHU de Reims, Reims, France, [6]CHU Reims genetics Unit, Reims, France, [7]SFR CAP SANTE, UFR de médecine, Reims, France, [8]EA3801, Reims, France.

Aarskog-Scott syndrome (ASS) or facio-digito-genital dysplasia (OMIM#305400) is a rare X-linked inherited disorder caused by pathogenic variants in the FYVE, RhoGEF, and pleckstrin homology domain-containing protein 1 (*FGD1*) gene. This syndrome affects about 1 in 1,000,000 births. Affected patients present a broad clinical phenotype with dysmorphism, short stature, skeletal and urogenital anomalies.

The probant is a two years old boy with camptodactyly, dysmorphism, cryptorchidism and short stature. Exome sequencing found a hemizygous variant c.123del (NM_004463) in *FGD1*, inherited from his mother who presents camptodactyly and hypertelorism. *FGD1* gene encodes the FGD1 protein, a guanine nucleotide exchange factors, able to activate Rho GTPase cell division cycle 42 (CDC42). Rho GTPase-CDC42 plays a role in control cytoskeleton-dependent membrane rearrangements, transcriptional activation, secretory membrane trafficking, extracellular matrix and cytoskeleton remodeling. Rho GTPases are involved in human diseases as developmental, hematological, autoinflammatory, autoimmune disorders. To our knowledge less than 60 damaging pathogenic variants in *FGD1* were reported to date, and the variant of our patient was not yet reported.

The aim of this work is to report the case of our patient and his family with a highly variable phenotype and to review the literature of patients in order to update the spectrum of this rare disease.

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P04.033.B Clinical and molecular study of a cohort of 79 patients with a pathogenic variation in *GDF5* gene and review of the literature

William Dufour[1], *Fabienne Escande*[1], *Anne-Sophie Jourdain*[1], *Odile Boute*[1], *Anne Dieux*[1], *Carherine Vincent-Delorme*[1], *Muriel Holder*[2], *Francesca Forzano*[3], *Laurence Faivre*[4], *Christel Thauvin*[4], *Marion Gerard*[5], *David Geneviève*[6], *Marjolaine Willems*[6], *Bertrand Isidor*[7], *Chloé Quélin*[8], *Sylvie Odent*[8], *Valérie Cormier-Daire*[9], *Geneviève Baujat*[9], *Gilles Morin*[10], *Lyse Ruaud*[11], *Yline Capri*[11], *Alice Goldenberg*[12], *Yves Alembik*[13], *Nicole Revençu*[14], *Bianca Ethel Gutiérrez-Amavizca*[15], *Nicolas Chassaing*[16], *Fanny Laffargue*[17], *Christine Francannet*[17], *Tiffany Busa*[18], *Véronique Paquis*[19], *Sylvie Manouvrier*[1], *Florence Petit*[1]

[1]CHU de Lille, Lille, France, [2]Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom, [3]Guy's and St Thomas' NHS Foundation Trust, London, France, [4]CHU de Dijon, Dijon, France, [5]CHU de Caen, Caen, France, [6]CHU de Montpellier, Montpellier, France, [7]CHU de Nantes, Nantes, France, [8]CHU de Rennes, Rennes, France, [9]Hôpital Necker-Enfants Malades, Paris, France, [10]CHU Amiens-Picardie, Amiens, France, [11]Hôpital Robert-Debré, Paris, France, [12]CHU de Rouen, Rouen, France, [13]CHU de Strasbourg, Strasbourg, France, [14]Cliniques Universitaires Saint-Luc, Brussels, Belgium, [15]Ciudad Juarez Autonomous University, Ciudad Juarez, Mexico, [16]CHU de Toulouse, Toulouse, France, [17]CHU de Clermont-Ferrand, Clermont-Ferrand, France, [18]Hôpital Universitaire Timone Enfants, Marseille, France, [19]CHU de Nice, Nice, France.

The *GDF5* gene is mainly expressed in the joints during the embryonic period. It is known to be responsible for different bone pathologies according to its alteration mechanism: brachydactyly in case of mono-allelic loss of function; symphalangism and multiple synostoses in case of heterozygous gain of function; Grebe, Du Pan and Hunter-Thompson syndromes in case of bi-allelic loss of function; susceptibility to osteoarthritis and hip dysplasia in the presence of polymorphisms within its promoter. Through the numerous associated phenotypes, *GDF5* illustrates the difficulties in interpreting genomic variations. We studied a large international cohort of 79 patients harbouring *GDF5* variations in order to: 1) Describe precisely the clinical and molecular data of patients; 2) Identify rare phenotypes and 3) Demonstrate genotype-phenotype correlations. We observed that some features seems to have a higher frequency in our cohort compared to those of the literature, notably 2nd ray clinodactyly and Angel Shaped Phalango-Epiphyseal Dysplasia, joint laxity, various dental abnormalities, premature osteoarthritis and hip disorders (mainly coxo-femoral dysplasia).

Table 1: Frequency of the different impairment in patients with brachydactyly type C

	cohort patients	percentage	literature patients	percentage
Angel-Shaped Phalango-Epiphyseal Dysplasia	22/63	34.9%	5/27	18.5%
1st rays brachymetacarpy	49/63	77.8%	16/27	59.3%
2nd rays brachymesophalangy	51/63	81%	18/27	66.7%
3rd rays brachymesophalangy	36/63	57.1%	13/27	48.1%
5th rays brachymesophalangy	47/63	74.6%	19/27	70.4%
2nd rays brachybasophalangy	14/63	22.2%	8/27	29.6%
3rd rays brachybasophalangy	14/63	22.2%	10/27	37%
2nd rays clinodactyly	22/66	33.3%	17/30	56.7%
3rd rays clinodactyly	14/66	21.2%	11/30	36.7%
5th rays clinodactyly	43/66	65.2%	17/30	56.7%
polyphalangy	19/63	30.2%	9/27	33.3%
pseudoepiphyses	16/63	25.4%	6/27	22.2%
foot damage	20/49	40.8%	11/26	42.3%
height<-2SD	21/57	36.8%	7/19	36.8%
bone age delay	24/26	92.3%	7/8	87.5%
dental anomalies	19/55	34.5%	1/15	6.7%
hip anomalies	15/61	24.6%	3/19	15.8%
joint laxity	16/62	25.8%	2/17	11.8%
premature osteoarthrosis	6/33	18.2%	0/14	0%
asymptomatic	3/74	4.1%	4/33	12.1%

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P04.034.C Porokeratosis of Mibelli in the Tunisian population: association of nonsense and synonymous variants

Haïfa El Mabrouk[1,2], *Lobna Boussofara*[3,4], *Hamza Chouk*[1,2], *Nadia Gharyani Fétoui*[3,4], *Ali Saad*[3,2], *Mohamed Denguezli*[3,4], *Dorra H'mida*[3,2]

[1]*Higher Institute of Biotechnology, Monastir, Tunisia,* [2]*Laboratory of Human Cytogenetics, Molecular Genetics and Reproductive Biology, Farhat HACHED University Hospital, Sousse, Tunisia,* [3]*Faculty of Medicine Ibn El Jazzar, Sousse, Tunisia,* [4]*Department of Dermatology and Venerology, Farhat HACHED University Hospital, Sousse, Tunisia.*

Introduction: Porokeratosis of Mibelli (PM) is a rare autosomal dominant genodermatosis linked to the *MVK* and *PMVK* genes. We aim to establish molecular evidence of PM in our Tunisian patients.

Material and methods: Direct sequencing was performed in a cohort of 8 patients with PM and 9 of their clinically healthy relatives from 2 unrelated families in central Tunisia.

Results: 7 patients and 2 healthy relatives presented a known pathogenic variant (*PMVK* c.412C> T; p.R138*), and 6 patients and 4 of their relatives presented a synonymous variant (*PMVK* c.147A>G; p.E49=).

Discussion: In the present work, we propose to report an association of the synonymous variant p.49Glu = in exon 2 to the pathogenic variant p.Arg138* at exon 4, both at the *PMVK* gene. This association seemed to modulate PM patients' phenotype. Patients carrying the association present either moderate phenotypes; the number of lesions exceeded 10 covering approximately 5% of the body surface or severe phenotypes: in which affected body surface exceeds 10%, with lesions of the genitals and the face. When the synonymous variant is absent, patients present only a few annular plaques with reduced size, suggesting a mild clinical phenotype.

Conclusion: The association between the two variants lead us to believe that the synonymous variant could constitute a modifier of the *PMVK* gene.

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P04.035.D GLI3-variants causing isolated polysyndactyly are not restricted to the gene's last third

Henrike L. Sczakiel[1,2], *Wiebke Hülsemann*[3], *Angela T. Abad-Perez*[1], *Sarina Schwartzmann*[1], *Denise Horn*[1], *Malte Spielmann*[4,5], *Stefan Mundlos*[1,2], *Martin A. Mensah*[1,6]

[1]*Charité - Universitätsmedizin Berlin, Institut für Medizinische Genetik und Humangenetik, Berlin, Germany,* [2]*Max Planck Institute for Molecular Genetics, RG Development & Disease, Berlin, Germany, Berlin, Germany,* [3]*Handchirurgie Kinderkrankenhaus Wilhelmstift, Hamburg, Germany,* [4]*Institut für Humangenetik Lübeck, Universität zu Lübeck, Lübeck, Germany,* [5]*Max Planck Institute for Molecular Genetics, Human Molecular Genomics Group, Berlin, Germany,* [6]*Berlin Institute of Health, Berlin, Germany.*

Introduction: Loss of function variants of *GLI3* are associated with a variety of forms of polysyndactyly: Pallister-Hall-Syndrome (PHS), Greig-Cephalopolysyndactyly-Syndrome (GCPS) and isolated polysyndactyly. So far, mutations in the first and third third of the *GLI3* gene have been associated with GCPS while mutations in the second third of *GLI3* have been associated with PHS. Cases of isolated polysyndactyly were attributed to mutations in the third third of *GLI3*. Here we present more than 30 individuals from over 15 families with pre- and postaxial polysyndactyly in whom we could confirm *GLI3* mutations.

Materials and Methods: Sanger sequencing of the *GLI3* gene was performed in patients with clinical findings suggestive for a *GLI3*-associated polysyndactyly syndrome. Additionally, we searched the literature for previously reported cases of either PHS, GCPS or isolated polysyndactyly with confirmed mutations in the *GLI3* gene.

Results: We tested over 80 individuals with polysyndactyly for *GLI3* variants and detected a causing mutation in more than 15 families. The mutations spanned almost the entire coding region of the *GLI3* gene (c.366 - c.4172) and were mostly amorphic. We observed no genotype-phenotype correlation within those cases. Overall, we found a high phenotypic variability within and across families. A close review of the literature revealed more cases of isolated polysyndactyly with mutations across nearly the entire coding region of *GLI3*.

Conclusions: We found that isolated polysyndactyly is a common phenotype of inherited as well as *de novo GLI3* mutations and is not restricted to mutations in the last third of the *GLI3* gene.

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P04.037.B A novel c.671_682del *NCSTN* variant in a family with Hidradenitis Suppurativa

Nikolai P. Pace[1], *Dillon Mintoff*[2], *Peter Bauer*[3], ***Isabella Borg***[1,2]

[1]*University of Malta, Msida, Malta,* [2]*Mater dei Hospital, Msida, Malta,* [3]*Centogene GmbH, Rostock, Germany.*

Introduction: Hidradenitis suppurativa (HS) is a chronic, suppurative condition of the pilosebaceous unit (PSU), the pathophysiology of which is still being elucidated. The disease is multifactorial, caused by both genetic and environmental factors. The potential role of underlying genetic variants, particularly within the γ-secretase complex has recently garnered significant research interest by the international HS community.

Methodology: The proband, a 21-year-old male and his 45-year-old mother had Hurley Stage IIIa HS and shared a follicular, Latent Class 2 phenotype. The maternal grandmother suffered from a mild form of axillary HS. In view of these findings, a familial form of HS with variable expressivity was suspected and molecular genetic studies were carried out.

Results: WES analysis on the proband revealed a novel heterozygous c.671_682del p. (Val224_Thr227del) variant in the Nicastrin (*NCSTN*) gene. This 12bp in-frame deletion lies in exon 6 of *NCSTN*, resulting in the loss of 4 amino acid residues. Targeted gene sequencing identified the same heterozygous variant in the affected mother. The *NCSTN* deletion was not identified in a local reference dataset comprising 60 ethnically matched whole exome sequences.

Conclusion: This result expands the mutational spectrum of the *NCSTN* gene. It is the first gene variant identified in HS Maltese patients. Further genetic studies will be carried out on all local HS patients to unravel the genetic aetiology of HS in the local population using a high throughput exome sequencing approach. We hope to establish genotype-phenotype associations which would lead to better clinical management and targeted treatment for HS.

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P04.038.C A missense mutation in *VAV3* in a familial case of high bone mass

Núria Martínez-Gil[1], *Diana Ovejero*[2], *Natalia Garcia-Giralt*[2], *Leonardo Mellibovsky*[2], *Xavier Nogués*[2], *Raquel Rabionet*[1], *Daniel Grinberg*[1], *Susanna Balcells*[1]

[1]*Department of Genetics, Microbiology and Statistics, Faculty of Biology, Universitat de Barcelona, CIBERER, IBUB, IRSJD, Barcelona, Spain,* [2]*Musculoskeletal Research Group, IMIM (Hospital del Mar Medical Research Institute), Centro de Investigación Biomédica en Red en Fragilidad y Envejecimiento Saludable (CIBERFES), ISCIII, Barcelona, Spain.*

Osteoporosis is the most common bone disease, characterized by a low bone mineral density (BMD) and increased risk of fracture. At the other end of the BMD spectrum, some individuals present strong, fracture-resistant, bones. Both osteoporosis and high bone mass (HBM) are heritable and their genetic architecture encompasses polygenic inheritance of common variants and some cases of monogenic highly penetrant variants in causal genes. We have investigated the genetics of a family presenting HBM segregating in an apparently Mendelian dominant pattern. Since polygenic causes and mutations in known HBM genes had been previously discarded, we searched for rare causal variants in novel genes by whole-exome sequencing in two affected and one non-affected family members. Rare coding variants fulfilling the inheritance pattern were further restricted to include only those genes that could be related to bone development, function or disease, leaving 8 variants. Of those, we highlight a missense variant in VAV3, a gene encoding a guanine-nucleotide-exchange factor with an important role in osteoclast activation and function. Although no previous cases of VAV3 mutations have been found in humans, Vav3 KO mice display dense bones, equivalent to the HBM phenotype present in our family. A second missense variant, which might play a secondary role, was found in SIK3. However, the mouse and human bone phenotypes associated with this gene do not fit with HBM. Future functional assessment of the VAV3 variant will likely confirm VAV3 as a novel HBM gene and a potential therapeutic target for osteoporosis. Funding: SAF2014-56562-R, SAF2016-75948-R.

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P04.039.D A new variant of c409delA in the *EXT2* gene identified among Yakut families with hereditary multiple exostosis

Alexandra E. Yakovleva[1], *Anastasia L. Danilova*[1], *Diana A. Petukhova*[1], *Polina I. Golikova*[1], *Gavril D. Moskvitin*[1,2], *Aitalina L. Sukhomyasova*[1,2], *Nadezda R. Maksimova*[1]

[1]*Research Laboratory "Molecular Medicine and Human Genetics", M. K. Ammosov North-Eastern Federal University, Yakutsk, Russian Federation,* [2]*Medical Genetic Center, Republican Hospital №1 - "National Medical Center", Yakutsk, Russian Federation.*

Hereditary multiple exostosis (HME) (OMIM 133700, OIMM 133701) is a genetically heterogeneous disease with an autosomal dominant mode of inheritance, characterized by the presence of multiple cartilaginous growths in the metaphyses of long bones.

Materials and Methods: We examined 27 unrelated probands with a clinical diagnosis of HME using mass parallel - massive for all *EXT1* and *EXT2* coding regions, followed by validation of the results by Sanger sequencing.

Results: Molecular genetic research revealed an unknown heterozygous frameshift variant c.409delA (p.Ile137fs) in the 2nd exon *EXT2* among 8 unrelated Yakut patients, which was also confirmed by direct Sanger sequencing. The detected mutation was checked in the Human Gene Mutation Database (HGMD), Leiden Open Variation Database (LOVD), Genome Aggregation Database (gnomAD). This mutation predicted to be highly damaging on a protein function. Experiments were performed using the equipment of the Center for collective use of the North-Eastern Federal University.

Conclusions: As a result of this study, for the first time in the Republic of Sakha (Yakutia), a previously undescribed heterozygous frameshift variant c.409delA (p.Ile137fs) in the 2nd exon of the *EXT2* gene. The study was supported by the Ministry of Science and Higher Education of the Russian Federation. (Project No. FSRG-2020-0014 "Genomics of Arctic: epidemiology, hereditary and pathology").

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P04.041.B Aberrant binding of mutant HSP47 affects post-translational modification of type I collagen and leads to osteogenesis imperfecta

Delfien Syx[1], *Yoshihiro Ishikawa*[2,3], *Jan Gebauer*[4], *Sergei P. Boudko*[5], *Brecht Guillemyn*[1], *Tim Van Damme*[1], *Sanne D'hondt*[1], *Sofie Symoens*[1], *Sheela Nampoothiri*[6], *Douglas B. Gould*[2], *Ulrich Baumann*[4], *Hans Peter Bächinger*[3], *Fransiska Malfait*[1]

[1]*Ghent University, Ghent, Belgium,* [2]*UCSF School of Medicine, San Francisco, CA, USA,* [3]*Oregon Health & Science University, Portland, OR, USA,* [4]*University of Cologne, Cologne, Germany,* [5]*Vanderbilt University Medical Center, Nashville, TN, USA,* [6]*Amrita Institute of Medical Sciences and Research Center, Cochin, India.*

Heat shock protein 47 (HSP47), encoded by the *SERPINH1* gene, is a molecular chaperone essential for correct folding of collagens. We report a homozygous p.(R222S) substitution in HSP47 in a child with severe osteogenesis imperfecta (OI). The highly conserved p.R222 residue is located within the collagen-interacting surface and HSP47-R222S shows a significantly reduced affinity for type I collagen in binding assays. This altered interaction leads to posttranslational overmodification of type I procollagen, with increased glycosylation and/or hydroxylation of lysine and proline residues as shown by mass spectrometry. Since a normal intracellular folding and secretion rate of type I procollagen was observed, this overmodification cannot be explained by prolonged exposure of the procollagen molecules to modifying enzymes, as is commonly observed in other OI types. We found significant upregulation of several molecular chaperones and enzymes involved in procollagen modification and folding on Western blot and RT-qPCR and showed that an imbalance in binding of HSP47-R222S to unfolded type I collagen chains results in increased binding of other chaperones and modifying enzymes in a gelatin sepharose pulldown assay. In conclusion, our findings suggest a compensatory mechanism for aberrant HSP47-R222S binding, eventually leading to overmodification of type I (pro)collagen chains, thereby underscoring the importance of HSP47 for proper posttranslational modification and providing insights into the molecular pathomechanisms of the p.(R222S) alteration in HSP47, which leads to a severe OI phenotype. This work was supported by Research Foundation Flanders (Belgium), Ghent University, German Research Council, National Institutes of Health and Shriners Hospitals for Children.

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P04.042.C Association of one-carbon metabolism-related genes and ichthyosis vulgaris manifestation in Eastern Ukraine

*Olena M. Fedota[1], **Iurii O. Sadovnychenko**[1,2], Halyna V. Makukh[3], Lilia B. Chorna[3], Larissa V. Roshcheniuk[2,4], Vitalii M. Vorontsov[4], Pavlo P. Ryzhko[4]*

[1]V.N. Karazin Kharkiv National University, Kharkiv, Ukraine, [2]Kharkiv National Medical University, Kharkiv, Ukraine, [3]Institute of Hereditary Pathology of National Academy of Medical Sciences of Ukraine, Lviv, Ukraine, [4]Regional Clinical Dispensary for Skin and Venereal Diseases no. 1, Kharkiv, Ukraine.

Introduction: The prevalence of ichthyosis vulgaris (IV) in Eastern Ukraine is 1:2557. The dermatosis is caused by *FLG* mutations R501X and 2282del4, but their penetrance in heterozygotes is incomplete. The purpose of the study was to analyze the effects of one-carbon metabolism-related genes on the IV clinical manifestation in *FLG* mutation carriers.

Materials and methods: The *MTHFR* C677T (rs1801133), *MTHFR* A1298C (rs1801131), *MTR* A2756G (rs1805087) and *MTRR* A66G (rs1801394) SNPs were analyzed by PCR-RFLP in 31 IV patients, 7 their non-IV first-degree relatives with *FLG* mutations and 150 healthy controls. Statistical analysis was performed using chi-square test and OR.

Results: In 2282del4/wt IV individuals, the distributions of wild-type, heterozygous and variant homozygous genotypes were: rs1801133 — 0.29:0.71:0.00; rs1801131 — 0.53:0.47:0.00; rs1805087 — 0.70:0.24:0.06; rs1801394 — 0.23:0.53:0.24. In this group the *MTR* 2756AA genotype and *MTR* 66GG genotype frequencies were 1.4-1.6 higher than in IV patients with the other *FLG* genotypes, the *MTR* 2756AA genotype frequency was 1.6 times higher than in healthy controls ($p < 0.01$). The association between IV and one-carbon metabolism-related genes was evaluated for single- and multilocus disease models. The strongest genotype-phenotype relationships were found for the genotypes: *MTHFR* 677CT — OR = 3.60 (95% CI 1.21−10.71, $p = 0.032$), *MTHFR* 677CT/*MTHFR* 1298AA +AC — OR = 4.39 (95% CI 1.47−13.14, $p = 0.008$), *MTHFR* 677CT/*MTHFR* 1298AA/*MTRR* 66AG — OR = 7.64 (95% CI 2.34−24.94, $p = 0.001$), *MTHFR* 677CT/*MTHFR* 1298AA/*MTR* 2756AA/*MTRR* 66AG — OR = 11.23 (95% CI 2.51−50.21, $p = 0.002$).

Conclusion: SNPs in one-carbon metabolism-related genes can be considered as modifiers of *FLG* 2282del4 mutation in IV clinical manifestation.

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P04.044.D Incontinentia pigmenti and favism due to a large genomic deletion including *IKBKG* and *G6PD*

***Maria Grossmann**[1], Stephanie Spranger[2], Snjezana Rendulic[3], Andrea Bier[1], Silke Reif[1], Manuela Timmer[1], Christian Jänecke[1], Vivien Klaschka[1], Jens Plaschke[1], Stefan Krüger[1]*

[1]Gemeinschaftspraxis für Humangenetik, Dresden, Germany, [2]Praxis für Humangenetik, Bremen, Germany, [3]Praxis für Humangenetik und Prävention, Stuttgart, Germany.

Incontinentia pigmenti (IP) is a genodermatosis in four stages including blistering, wart-like rash, swirling macular hyperpigmentation and linear hypopigmentation, and also affects hair, teeth, nails, eyes and brain. IP is caused by mutations in the *IKBKG* (*NEMO*) gene and inherited in an X-linked dominant manner with high penetrance. Affected females show a highly variable phenotype. IP is usually prenatally lethal in males. About 65% of females with IP have a recurrent ~11.7-kb deletion spanning exons 4 to 10 and ~8.6% have point mutations. Only seven other deletions were described to date.

Here we describe a 24-year-old female patient with typical skin lesions since birth. She showed neither neurological nor other symptoms of IP and suffered two miscarriages. Furthermore, the patient had intolerance of fava beans and some drugs, indicating X-linked dominant *G6PD* associated favism. Her mother and her sister were also affected of IP and favism.

Neither long-range (gap) PCR for detection of the recurrent exon 4 to 10 deletion, nor sequence analysis detected a pathogenic mutation. MLPA revealed a heterozygous deletion spanning the whole *IKBKG* gene as well as the 5′ adjacent entire *G6PD* gene. NC_000023.10:g.(153,744,322_153,759,773)_(153,793,401_153,798,250)del. This deletion is to our knowledge of yet undescribed extent. The only two patients with large *IKBKG* deletions involving the whole *G6PD* gene described so far by Fusco et al. did not show any clinical manifestations of *G6PD* deficiency in contrast to the patients described here.

In summary, this is the first description of a contiguous gene syndrome including Incontinentia pigmenti and favism.

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P04.045.B The importance of extracutaneous organ involvement for the clinical severity and prognosis observed in incontinentia pigmenti caused by *IKBKG* mutations

*Hwa Young Kim, **Jung Min Ko**, Hyun Beom Song, Kyu Han Kim, Jong Hee Chae, Man Jin Kim, Moon-Woo Seong*

Seoul National University Hospital, Seoul, Korea, Republic of.

Incontinentia pigmenti (IP) is a rare X-linked skin disease caused by mutations of the *IKBKG* gene, which is required for activation of the nuclear factor-kappa B signaling pathway. Multiple systems can be affected with highly variable phenotypic expressivity. We aimed to clarify the clinical characteristics observed in molecularly-confirmed Korean IP patients. Medical records of 25 females confirmed as IP by molecular genetic analysis were retrospectively reviewed. A phenotypic score of extracutaneous manifestations was calculated to assess the disease severity. The *IKBKG* gene partial deletion or intragenic mutations were investigated by a long-range PCR, multiplex ligation-dependent probe amplification, and direct sequencing methods. Among 25 individuals, 18 (72%) were sporadic cases. All patients showed typical skin manifestations at inborn or during the neonatal period. Extracutaneous findings were noted in 17 (68%) cases; ocular manifestations (28%), neurological abnormalities (28%), hair abnormalities (20%), dental anomalies (12%), nail dystrophy (8%). The common *IKBKG* exon 4-10 deletion was observed in 20 (80%) patients. In addition, 5 intragenic sequence variants were identified, including 3 novel ones. The phenotype scores were highly variable from abnormal skin pigmentation only to one or more extracutaneous features, even though there was no meaningful significant difference for each clinical characteristic between the groups with sequence variants and common large deletion. Heterogeneity of

extracutaneous manifestations and high incidence of sporadic cases were observed in our cohort with IP. Long-term monitoring with multidisciplinary management is essential for evaluating the clinical status, providing adequate genetic counseling and understanding genotype-phenotype correlation in IP.

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P04.047.D Further insights in the *FKBP14*-related khyphoscoliotic Ehlers-Danlos syndrome: report of 3 unrelated individuals and 2 new pathogenic variants

Marlies Colman[1,2], *Robin Vroman*[1,2], *Tibbe Dhooge*[1,2], *Zoë Malfait*[1,2], *Biruté Burnyté*[3], *Sheela Nampoothiri*[4], *Delfien Syx*[1,2], *Fransiska Malfait*[1,2]

[1]*Ghent University, Ghent, Belgium,* [2]*Center for Medical Genetics, Ghent, Belgium,* [3]*Vilnius University Hospital, Vilnius, Lithuania,* [4]*Amrita Institute of Medical Sciences & Research Centre, Kerala, India.*

The kyphoscoliotic type of Ehlers-Danlos syndrome (kEDS) is an autosomal recessive connective tissue disorder characterized by kyphoscoliosis, muscle hypotonia and generalized joint hypermobility. It results from deficiency of either lysyl hydroxylase 1 (LH1 encoded by *PLOD1*) or the peptidyl-prolyl *cis-trans* isomerase family FK506-binding protein 22kDa (FKBP22 encoded by *FKBP14*). FKBP22 acts as a molecular chaperone involved in the folding and quality control of collagen molecules (among which types III and VI collagen). Deficiency of FKBP22 may lead to accumulation of incorrectly folded collagen molecules in the endoplasmic reticulum (ER), causing premature interactions. We present the clinical manifestations of three non-related individuals with homozygous pathogenic *FKBP14* variants: proband 1 (c.587A>G; p.(Asp196Gly)); proband 2 (c.362dupC; p.(Glu122Argfs*7)) and proband 3 (c.2T>G; p.(Met1?)); with experimental data of the variants found in probands 1 and 2. Both the c.587A>G; p.(Asp196Gly) and the c.362dupC; p.(Glu122Argfs*7) variant cause absence of FKBP22 protein. In addition, we found intracellular accumulation of types III and VI collagen and impaired fibroblast migration. Investigation of ER-stress related proteins (CHOP, XBP1, ATF6, (p)eIF2aplha, BIP) did not reveal any significant upregulation.

Conclusion: This study broadens the clinical and molecular spectrum of *FKBP14*-related kEDS with three non-related individuals and two new pathogenic variants. We showed delayed fibroblast migration and intracellular accumulation of type III and type VI collagen and we provide the first evidence of a homozygous pathogenic missense variant.M.C., D.S. and F.M. are a PhD candidate, a postdoctoral researcher and a senior clinical investigator respectively, of the Research Foundation Flanders, Belgium.

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P04.048.C Homozygosity for a previously unreported deletion in B3GAT3 causes linkeropathy - a clinical report describing an adult patient

Anneli C. S. Bolund*, Bente Langdahl, Trine B. Laurberg, Michel B. Hellfritzsch, Hans Gjørup, Bjarne Møller-Madsen, Trine Ø. Nielsen, Stense Farholt, Pernille A. Gregersen*

Aarhus University Hospital, Aarhus N, Denmark.

Background: Proteoglycans (PGs) are complex macromolecules consisting of a core protein and glycosaminoglycan (GAG) side chains. PGs are important for the constitution and functioning of the connective tissue. The normal composition of the GAG side chains defines the nature of the PGs and a wide range of biological events. Deficiencies of specific enzymes involved in the linkage of GAGs to the core protein, leads to a heterogeneous disease group called Linkeropathies. This is a group of multisystem conditions characterized by skeletal dysplasia and various extra-skeletal features. The conditions show variable severity and overlapping phenotypes. β-1,3-glucuronyltransferase 3, encoded by *B3GAT3*, is an enzyme involved in the linkage process to form functional PGs, and biallelic pathogenic variants in *B3GAT3* hence leads to linkeropathy.

Case presentation: A now 22-year-old female patient, born of consanguineous parents, is presented. The disease history included congenital severe joint malalignment of elbows, hips, knees and feet, continuous hypermobility, severe kyphoscoliosis, osteoporosis with multiple fractures, congenital diaphragmatic hernia, and mild dysmorphic features. Whole exome sequencing was performed, and homozygosity for a novel in-frame deletion in *B3GAT3*, (c.61_63delCTC (p.(Leu21del))) was detected. Both unaffected parents (first double cousins) were heterozygous carriers.

Conclusion: This is the first report to describe homozygosity for an in-frame deletion in *B3GAT3* (p.(Leu21del)) and the first adult phenotype described. Previously described cases of *B3GAT3*-deficiency were all children with phenotypes ranging from prenatal manifestation and early lethality to less severe. We suggest that this novel homozygous in-frame deletion in *B3GAT3* causes a recessive form of linkeropathy.

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P04.049.B Lipoid proteinosis: A novel mutation in *ECM1* gene in a Turkish patient

Elifcan Tasdelen[1], *Isa An*[2]

[1]*Department of Medical Genetics, Sanliurfa Education and Research Hospital, Sanlıurfa, Turkey,* [2]*Department of Dermatology, Sanliurfa Education and Research Hospital, Sanlıurfa, Turkey.*

Background: Lipoid proteinosis (LP) (OMIM-247100), also known as Urbach-Wiethe disease caused by loss-of-function mutations in the *ECM1* gene, is characterized by skin lesions such as yellow infiltrating papules, acneiform scars and verrucous hyperkeratosis. In most patients, hoarse voice is observed due to vocal cord thickening. All these findings occurs as a result of accumulation of hyaline-like material in the skin and mucosa. Furthermore, neuropsychiatric findings have been associated with intracranial calcifications observed in LP patients. Extracellular matrix protein-1 (ECM1) encoded by *ECM1* gene is involved in differentiation of keratinocytes by binding to structural proteins, and angiogenesis. **Case report:** Here, we desribe 7-year-old girl, a child of consanguineous parents, with diffuse acneiform scar on the face, moniliform blepharosis on the eyelids, verrucous lesions on dorsum of the hands, hypopigmented patches on the arms and back and hoarse voice. In sequencing analysis of *ECM1* gene, a novel homozygous NM_004425:c.1246 C>T(p.Arg416Ter) mutation in exon 8 causing premature stop codon was detected. This variant neither found in ExAC nor 1000G databases. The effect of the mutation on protein is predicted as damaging by in silico analysis.

Conclusion: Approximately sixty mutations associated with LP have been reported so far. Half of these mutations have been detected in exons 6 and 7 of the *ECM1* gene. The p.Arg416Ter

mutation is outside these hotspot regions and have not been reported previously. It explains the phenotype of our patient likely by causing nonsense mediated decay resulting in the non-functional ECM1 protein.

E. Tasdelen: None. **I. An:** None.

P04.050.C Novel variant in *SMAD3* gene associated with Loeys-Dietz syndrome type 3

Albertas Valintėlis[1], *Marius Šukys*[1], *Eglė Ereminienė*[2], *Inga Nasvytienė*[1], *Lina Basel-Salmon*[3,4,5], *Rasa Ugenskienė*[1]

[1]*Department Of Genetics And Molecular Medicine, Lithuanian University of Health Sciences, Kaunas, Lithuania,* [2]*Department of Cardiology, Lithuanian University of Health Sciences, Kaunas, Lithuania,* [3]*Raphael Recanati Genetics Institute, Rabin Medical Center, Beilinson Hospital, Petah Tikva, Israel,* [4]*Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel,* [5]*Laboratory of Immunology and Genetics, Felsenstein Medical Research Center, Petah Tikva, Israel.*

Introduction: Loeys-Dietz syndrome (LDS) is a systemic connective tissue disorder characterized by vascular findings, skeletal abnormalities, craniofacial features and cutaneous findings which sometimes can be misdiagnosed as Marfan syndrome The diagnosis is established based on clinical findings and the identification of heterozygous pathogenic variant in one of the several reported genes.

Methods: We report a 41-year-old woman with a prior clinical diagnosis of Marfan syndrome (systemic score 8 and aortic root dilatation Z score 2.99). Additionally she had right vertebral artery tortuosity, a history of gastric hemorrhage from Dieulafoy's ulcer, hand osteoarthritis, osteoporosis (repeated spontaneous metatarsal fractures), hypermobile joints (Beighton score 4), uvula aplasia, velvety and translucent skin with visible underlying veins. The patient also has primary thrombocythemia (with *JAK2* pathogenic variant) and chronic tubotympanic suppurative otitis media. No affected relatives were documented.

Results: We performed cardiovascular gene panel analysis via Next generation sequencing: no *FBN1* gene variant was found, but heterozygous gene *SMAD3* variant NM_005902.4: c.1262G>A of unknown significance was detected. This variant is not found in control databases and was not reported in literature, in silico tools predict it as deleterious, UniProt database suggest that an important protein domain is affected. Variant segregation analysis was not performed as patient's parents are deceased. As clinical features are more suggestive to Loeys Dietz type 3 syndrome and variant is predicted deleterious we determined the variant as likely pathogenic.

Conclusion: Genetic testing allowed us to establish a proper diagnosis and provide more extensive management plan for the patient.

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P04.051.D Four hypotrichosis families with pathogenic variants in the gene *LSS* presenting with and without neurodevelopmental phenotypes

Nicole Cesarato[1], *Maria Wehner*[1], *Mari Ghughunishvili*[2], *Axel Schmidt*[1], *Michael Lentze*[3], *Cristina Has*[4], *Matthias Geyer*[5], *F. Buket Basmanav*[1], *Regina C. Betz*[1]

[1]*Institute of Human Genetics, University of Bonn, Bonn, Germany,* [2]*Givi Zhvania Academic Clinic of Pediatrics, Tbilisi State Medical University, Tbilisi, Georgia,* [3]*Department of Pediatrics, University of Bonn, School of Medicine & University Hospital Bonn, Bonn, Germany,* [4]*Department of Dermatology, University Hospital Freiburg, Freiburg, Germany,* [5]*Institute of Structural Biology, University of Bonn, School of Medicine, Bonn, Germany.*

Introduction: *LSS*, encoding for lanosterol synthase, has been associated with congenital cataract, autosomal recessive hypotrichosis simplex resp. congenital alopecia with or without neuroectodermal phenotypes including intellectual disability, epilepsy, microcephaly, genital abnormalities in males and dermatological symptoms. In this study, we report novel pathogenic variants found in *LSS*.

Materials and Methods: Direct Sanger sequencing of the *LSS* gene or Whole Exome Sequencing was performed on individuals affected with hypotrichosis and their family members. Protein structure modelling was used to reveal the consequences of the identified mutations.

Results: All affected individuals, originating from Iraq, Syria, Afghanistan and Georgia, suffered from mild hypotrichosis to severe alopecia. One patient presented also with a developmental speech disorder, learning difficulties and microcephaly. The following variants were found either in homozygous or in compound heterozygous state and co-segregated in these families: c.530G>A; p.(Arg177Gln), c.934C>T;p.(Arg312Trp), c.881G>T;p.(Arg294Leu), c.1702C>T;p.(Arg568Trp). The most interesting variant was the homozygous synonymous variant c.393G>A;p.(131Leu=), shown to activate a cryptic donor splice site, resulting in an in frame deletion of 11 amino acid residues (r.425-457del). Modelling of the mutant sites based on the crystal structure of LSS revealed that all mutations have consequences on the 3D protein structure.

Conclusions: It remains unclear which variants in *LSS* lead to an alopecia phenotype only, and which lead to accompanying severe neurodevelopmental and dermatological phenotypes. If significantly more patients with mutations in *LSS* will be reported, we may be able to develop a better understanding of this protein and allow predictions concerning the phenotypic outcomes of individual *LSS* mutations.

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P04.052.A Searching for *TGFB3* gene mutations among Polish patients with Marfan syndrome and related disorders

Maria Pilarska-Deltow[1], *Marzena Skrzypczak-Zielińska*[2], *Anna Junkiert-Czarnecka*[1], *Aneta Bąk*[1], *Marta Heise*[1], *Olga Haus*[1]

[1]*Nicolaus Copernicus University, Torun, Poland,* [2]*Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland.*

Introduction: Marfan syndrome (MFS) is the best known congenital disease of connecting tissue. The classical symptoms of MFS include skeletal, cardiovascular and eye abnormalities. However, the variety and variable severity of them make the diagnosis difficult due to the similarities with Marfan-like syndromes, including Loeys-Dietz syndrome (LDS). According to the classification, there are six subtypes of LDS. Mutations in *TGFB3* are responsible for LDS 5, which is less symptomatic and without molecular analysis can easily be confused with MFS.

Materials and Methods: Sanger sequencing was performed for 119 Polish patients with suspicion of Marfan or another Marfan-like syndrome.

Results: Sequencing of *TGFB3* revealed three mutations. According to VarSome database, mutation c.412G>T (p.Ser138Ala), with the allele frequency 0.01% (ExAc) was predicted as benign. Mutation c.488G>A (p.Arg163Gln), with the allele frequency

0.004% (ExAc) was classified as a variant of uncertain significance (VUS) (VarSome). The frequency of the mutation c.1138C>T (p.Pro380Ser) is not available in the ExAc, according to *in silico* tools this is also VUS. In the case of this mutation (p.Pro380Ser) we confirmed the paternal inheritance of the mutation in the parental study.

Conclusions: Mutations were detected in patients in whom previous NGS examination of the common genes associated with Marfan-like syndromes did not reveal pathogenic variants. The results point at the need to study less obvious genes of marfanoid syndromes to improve their diagnostics, which may have implications for patient prognosis, as well as diagnosis and prevention in family members.The investigation was supported by Nicolaus Copernicus University grant WL174.

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P04.053.B Targeted next generation sequencing substantially advances molecular diagnosis of Marfan and Marfan related disorders

Darina L. Kachakova[1], *Kunka Kamenarova*[1], *Kalina Mihova*[1], *Ivo Kremensky*[1], *Ivanka Dimova*[2], *Vanyo Mitev*[1], *Radka Kaneva*[1]

[1]*Laboratory of Genomic Diagnostics, Molecular Medicine Center, Department of Medical Chemistry and Biochemistry, Medical Faculty, Medical University of Sofia, Sofia, Bulgaria,* [2]*Laboratory of Genomic Diagnostics, Molecular Medicine Center, Department of Medical Chemistry and Biochemistry, Medical Faculty, Medical University of Sofia, 2- Department of Medical Genetics, Medical Faculty, Medical University of Sofia, Sofia, Bulgaria.*

Introduction: There are more than 200 heritable connective tissue disorders. Marfan syndrome (MFS) is a rare, autosomal-dominant connective tissue multisystem disorder. Significant clinical overlap with other systemic connective tissue diseases, has been documented.

Materials and methods: Eight patients with differential diagnosis Marfan and Marfan like syndromes and 1 patient with Ehlers-Danlos were directed for targeted next generation sequencing (NGS) in Molecular medicine center in the period 2019-2020 year. NGS was performed on MiSeq platform using TruSight one kit. Direct sequencing by Sanger was used in order to confirm estimated pathogenic variants.

Results: Genetic cause of the disease was estimated in 4 from 9 analyzed patients. In two patients *FBN1* mutations were found (c.8051+2T>A and p.Arg516Ter). In the other patients the following pathogenic variants were detected: c.1877A>T (p.Lys626Met) in *SKI* and c.1642A>C (p.Ser548Arg) in *SOS1* and thus clinical diagnoses were refined. In three patients we found VUS in the following genes: *COL5A2*, *COL11A1*, *FLNB*, *NOTCH1*, *BAG3*. In one patient with aortic aneurism a new likely pathogenic variant c.779delC (p.Pro260Hisfs*47) in *FOXE3* was found. Pathogenic mutations in forkhead domain of this gene were previously linked to autosomal dominant thoracic aortic aneurism. Although the new variant lies outside this domain we suggest that is still possible to contribute to the disease.

Conclusion: Many connective tissue disorders have overlapping symptoms and targeted NGS sequencing contributes substantially to genetic diagnosis and refinement of the clinical diagnosis in some of the cases. This work was supported by infrastructural projects D01-285/2019; D01-395/2020 funded by MES.

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P04.054.C Four novel families expand the genotypic and phenotypic landscape of *MESD*-related Osteogenesis Imperfecta

Thao Tran[1], *Rachel Keller*[1], ***Brecht Guillemyn***[2], *Melanie Pepin*[1], *Jane Corteville*[3], *Samir Khatib*[4], *Mohammad-Sadegh Fallah*[5], *Sirous Zeinali*[5,6], *Fransiska Malfait*[2], *Sofie Symoens*[2], *Paul Coucke*[2], *Peter Witters*[7], *Hamideh Bagherian*[4,5], *Deborah Nickerson*[1], *Michael Bamshad*[1], *Jessica Chong*[1], *University of Washington Center for Mendelian Genomics, Peter Byers*[1]

[1]*University of Washington, Seattle, WA, USA,* [2]*Ghent University, Ghent, Belgium,* [3]*Case Western Reserve University, Cleveland, OH, USA,* [4]*GMDC Al Quds University, Al Quds, Palestinian Territory,* [5]*Kawsar Human Genetics Research Center, Tehran, Iran, Islamic Republic of,* [6]*Pasteur Institute of Iran, Tehran, Iran, Islamic Republic of,* [7]*University Hospital Leuven, Leuven, Belgium.*

The bone disorder osteogenesis imperfecta (OI) is genetically heterogeneous. Most affected individuals have an autosomal dominant disorder caused by heterozygous variants in either of the type I collagen genes (*COL1A1* or *COL1A2*). Rare autosomal recessive forms of OI are associated with variants in genes that encode proteins involved in the modification of collagens, regulation of collagen expression, alteration of cellular differentiation along the bone lineage, and in collagen secretion. Recently, four different biallelic pathogenic variants in Mesoderm Development LRP Chaperone (*MESD*) were shown to cause a progressively deforming recessive type of OI, associated with recurrent fractures and oligodontia in five patients of five families. We report four additional patients from four independent families with biallelic variants in *MESD*: the earlier reported c.632dupA p.(Lys212-Glufs*19) and c.676C>T p.(Arg226*) - which are associated with a severe form of OI - and one new pathogenic variant c.603-606delTAAA (p.Asn201Lysfs*15) which causes a neonatal lethal form of OI. MESD acts in the WNT signaling pathway where it is thought to play a role in the folding of the WNT co-receptors Low Density Lipoprotein Receptor-Related Proteins 5 and 6 (LRP5 and LRP6) and in chaperoning their transit to the cell surface. We hypothesize that in the absence of functional MESD, WNT signaling in osteogenic cells is blocked. Our report broadens the phenotypic and genetic spectrum of *MESD*-related OI, provides additional insight into the pathogenic pathways and underscores the necessity of MESD for normal WNT signaling and bone formation.

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P04.055.D Sixth family with confirmed metaphyseal dysplasia, Spahr type and a novel variant in *MMP13*: case report and review of the literature

André M. Travessa[1], *Silvia Modamio-Høybjør*[2], *Karen E. Heath*[2], *Ana Berta Sousa*[1]

[1]*Medical Genetics Department, Department of Pediatrics, Hospital de Santa Maria, Centro Hospitalar Universitário Lisboa Norte, Lisbon, Portugal,* [2]*Institute of Medical and Molecular Genetics (INGEMM), Hospital Universitario La Paz, Universidad Autonóma de Madrid, IdiPAZ, CIBERER, ISCIII, Skeletal Dysplasia Multidisciplinary Unit (UMDE), Hospital Universitario La Paz, and ERN-BOND, Madrid, Spain.*

Introduction: Metaphyseal dysplasia, Spahr type (MDST, MIM#250400) is an ultra-rare metaphyseal dysplasia likely often mistaken as rickets. Only 16 patients from eight families have been reported, with molecular confirmation in five families (10 patients). We present a case of MDST and review the existing literature, highlighting the features that differentiate this from other metaphyseal dysplasias.

Case report: The proband is a 23-month-old gild referred for growth delay and *genu varum*. She was born at 33 weeks of gestation to healthy unrelated parents with no relevant family history. At birth, she had normal somatometry for gestational age. The neonatal period was unremarkable. *Genu varum* was noted at 6 months. Length crossed centiles downwards and was -2.75 SD at 19 months. She was otherwise healthy, and had a normal psychomotor development. The analytical evaluation of calcium and phosphate metabolism was normal, excluding rickets. However, the skeletal survey showed metaphyseal irregularities in the long bones, especially the lower limbs, compatible with a metaphyseal dysplasia. A customized skeletal dysplasia NGS panel was thus performed, and the pathogenic variant c.287_293del, p.(Cys96Phefs*19) in *MMP13* gene was identified in apparent homozygosity, confirming the diagnosis of MDST.

Discussion and conclusions: We report the 11th patient (sixth family) with confirmed MDST, and review the existing literature. Patients with MDST present with postnatal mild short (or even normal) stature. *Genu varum* is milder and knee pain is more common as compared to other metaphyseal dysplasias. No extra-skeletal features or laboratory findings exist. NGS is the investigation of choice for MDST.

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P04.057.B Clinical utility of a sponsored, no-cost skeletal dysplasia gene panel testing program: Results from 850 tests

Guillermo Seratti[1], *Vikram Pansare*[1], *Tiffany Yar Pang*[1], *Emanuela Izzo*[1], *William Mackenzie*[2], *Cathleen Raggio*[3], *Klane White*[4], *Rebecca Truty*[5], *Britt Johnson*[5], *Swaroop Aradhya*[5]

[1]*BioMarin Pharmaceutical Inc, Novato, CA, USA,* [2]*Nemours Alfred I. duPont Hospital for Children, Wilmington, DE, USA,* [3]*Hospital for Special Surgery, Uniondale, NY, USA,* [4]*Seattle Children's Hospital, Seattle, WA, USA,* [5]*Invitae, San Francisco, CA, USA.*

Rare diseases, such as mucopolysaccharidosis (MPS) IVA (Morquio A syndrome) and MPS VI (Maroteaux-Lamy syndrome), are often misdiagnosed as other types of skeletal dysplasia (SD) or may go undiagnosed for extended periods, potentially resulting in delayed intervention and irreversible disease progression. Discover Dysplasias™ sponsored testing program offers a focused SD gene panel for US and Canadian patients, with the goal of helping facilitate timely diagnoses. Eligible patients must have one or more of: skeletal abnormalities suggestive of SD, short stature, disproportionate growth, dysmorphic facial features or other signs suggestive of SD. The program uses a panel with 109 genes associated with SD. Third-party reflex biochemical enzyme testing is offered for inconclusive MPS molecular results. Genetic counseling is provided for all patients as part of the program. A total of 850 US patients were tested under the program from December 2019-August 2020. Median age at testing was 7 years (range: 0-90). Initial symptom onset was noted prenatally for 22.7% and at birth for 32.9%. Median age at first sign among patients presenting after birth was 5 years. A genetic diagnosis was established in 210 patients (36 genes): a molecular diagnostic yield of 24.7%. One MPS VI patient and two MPS IVA patients were identified; the latter were confirmed by reflex enzyme testing. Use of a targeted gene panel testing program, with genetic counseling support and MPS reflex enzyme testing, has clinical utility in identifying the genetic etiology of MPS and SD, and may allow for disease-specific interventions and proactive treatment plans.

G. Seratti: A. Employment (full or part-time); Significant; BioMarin Pharmaceutical Inc. E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; BioMarin Pharmaceutical Inc. **V. Pansare:** A. Employment (full or part-time); Significant; BioMarin Pharmaceutical Inc. E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; BioMarin Pharmaceutical Inc. **T.Y. Pang:** A. Employment (full or part-time); Significant; BioMarin Pharmaceutical Inc. E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; BioMarin Pharmaceutical Inc. **E. Izzo:** A. Employment (full or part-time); Significant; BioMarin Pharmaceutical Inc. E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; BioMarin Pharmaceutical Inc. **W. Mackenzie:** D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); Modest; BioMarin Pharmaceutical Inc. F. Consultant/Advisory Board; Modest; LPA, BioMarin Pharmaceutical Inc, MPS. Other; Modest; BioMarin Pharmaceutical Inc. **C. Raggio:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; BioMarin Pharmaceutical Inc, NextCure, OIF. D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); Modest; BioMarin Pharmaceutical Inc, Alexion. F. Consultant/Advisory Board; Modest; OIF, EDS, SDMC, BioMarin Pharmaceutical Inc, Ascendis, Alexion, Mereo. **K. White:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; BioMarin Pharmaceutical Inc, Ultragenyx, Ascendis, Theracon. D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); Modest; BioMarin Pharmaceutical Inc, Ultragenyx. F. Consultant/Advisory Board; Modest; National MPS Society, Little People of America, BioMarin Pharmaceutical. Other; Modest; UptoDate.com. **R. Truty:** A. Employment (full or part-time); Significant; Invitae. E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; Invitae. **B. Johnson:** A. Employment (full or part-time); Significant; Invitae. E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; Invitae. **S. Aradhya:** A. Employment (full or part-time); Significant; Invitae. E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; Invitae.

P04.058.C Exome sequencing combined with RNA sequencing clarifies the mode of inheritance of MYH3-associated spondylocarpotarsal syndrome: a case report

Marija Volk, *Karin Writzl, Matevž Jus, Aleš Maver, Helena Jaklič, Borut Peterlin*

Clinical institute of genomic medicine, UMC Ljubljana, Ljubljana, Slovenia.

Introduction: Pathogenic variants in the MYH3 gene have been associated with dominant and recessive Contractures, pterygia, and spondylocarpotarsal fusion syndrome, type 1 (CPSFS1). By combining exome and RNA sequencing, we provide evidence that MYH3:c.1581+1G>A variant, previously reported in association with apparently recessive CPSFS1 (PMID:29805041) is likely associated with a dominant predisposition to CPSFS1.

Materials and methods: A 14-month girl was referred to our institute with scoliosis, contractures, ptosis, short stature, bicuspid aortic valve and suspected skeletal dysplasia. Familial history revealed that the mother has short stature, scoliosis, contracture

of Vth finger and ptosis, while maternal uncle and grandfather have short stature and scoliosis. We performed exome sequencing (ES) and cDNA sequencing of the targeted MYH3 region.

Results: ES revealed the presence of a heterozygous pathogenic variant MYH3(NM_002470.4):c.1581+1G>A in the proband. Because this variant was originally reported in association with recessive inheritance, it was not initially considered causative in heterozygous form. However, segregation analyses revealed that the similarly affected mother also carried the identified variant as affected proband. Expression analysis of mRNA MYH3 transcript showed retention of intron 15, which was predicted to lead to an in-frame insertion of 34 amino-acid sequences. This insertion possibly leads to a gain-of-function effect.

Conclusion: We demonstrate that MYH3 variant c.1581+1G>A is an in-frame insertion and might also be associated with the dominant type of CPSFS1. Our case highlights the utility of expression analysis, which is essential for the correct interpretation of splice-site variants and genetic counselling.

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P04.059.D Myhre Syndrome: a rare cause of short stature and osteomuscular pain

Antonio Miguel Poyatos-Andújar[1], *Susana García-Linares*[1], *Margarita Martínez-Atienza*[2], *Francisca Quintana-Luque*[1], *Susana Pedrinaci-Rodríguez*[2], *María Luz Bellido-Díaz*[2], *Matías Pérez-Sánchez*[2]

[1]*Hospital Universitario Clínico San Cecilio, Granada, Spain,* [2]*Hospital Universitario Virgen de las Nieves, Granada, Spain.*

Introduction: Myhre syndrome is a rare autosomal dominant connective tissue disorder with multisystem involvement characterized by a clinical spectrum that includes growth retardation, skeletal anomalies, muscular hypertrophy, joint stiffness, facial dysmorphism, deafness, cardiovascular disease, learning and social challenges and abnormal sexual development. The molecularly confirmed cases have a de novo heterozygous gain-of-function mutation in SMAD4.

Material and Methods: We present a 20-years-old girl who was born after a dichorionic diamniotic twin pregnancy with increased nuchal translucency for this fetous. The patient present at birth low weight and increased pulmonary pressures, during her development present slow weight gain, muscular weakness, hearing loss, challenging behavior, scoliosis and menstrual dysfunction. All molecular and citomolecular studies performed during the infancy were normal. We performed a clinical exome sequencing (CES) analysis after clinical and familiar evaluations.

Results: We found the pathogenic variant c.1486C>T, p. Arg496Cys in the SMAD4 gene, this variant was determined as *de novo* with the segregation analysis.

Conclusions: In our case, the diagnosis was delayed beyond the second life decade, this illustrates the difficulty of correctly diagnosing patients with Myhre syndrome. The frequency of neoplasia in series of cases and endometrial cancer in patients with Myhre syndrome, raises the possibility of cancer susceptibility in these patients. It is important to alert clinicians to the possibility of detecting this syndrome for a correct treatment during infancy and surveillance during adult age.

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P04.060.A Large deletions of *NF1*: phenotypical description and parental origins of a severe condition

Laurence Pacot[1,2], *Dominique Vidaud*[1,2], *Audrey Sabbagh*[3], *Ingrid Laurendeau*[2], *Audrey Briand-Suleau*[1,2], *Audrey Coustier*[1], *Théodora Maillard*[1], *Cécile Barbance*[1], *Patrick Nitschké*[4], *Arnaud Jannic*[5], *Salah Ferkal*[5], *Béatrice Parfait*[1,2], *Michel Vidaud*[1,2], *members of the NF France Network, Pierre Wolkenstein*[5], ***Eric Pasmant***[1,2]

[1]*Service de Génétique et Biologie Moléculaires, Hôpital Cochin, DMU BioPhyGen, AP-HP.Centre-Université de Paris, Paris, France,* [2]*Institut Cochin, Inserm U1016 - CNRS UMR8104 - Université de Paris, CARPEM, Paris, France,* [3]*UMR 261 MERIT, IRD, Université de Paris, Paris, France,* [4]*Bioinformatics Platform, Imagine Institute, INSERM UMR 1163, Université de Paris, Paris, France,* [5]*Département de Dermatologie, AP-HP and Université Paris 12, Hôpital Henri-Mondor, Créteil, France.*

Background: Whole *NF1* locus deletions are identified in 5-10% of patients affected by neurofibromatosis type 1 (NF1). Several studies have previously described particularly severe forms of the disease in NF1 patients with *NF1* deletion. However, comprehensive descriptions of large cohorts are still missing to fully characterize this contiguous gene syndrome.

Methods: NF1 patients from a large French NF1 cohort were molecularly characterized using next-generation sequencing, microsatellites analysis, and MLPA between 2005 and 2020. *NF1*-deleted patients were enrolled and phenotypically characterized with a standardized questionnaire. Parental origin of *de novo NF1* deletions was determined in thirty trios and three duos using four intragenic and three extragenic microsatellites in the *NF1* locus.

Results: A deletion of the *NF1* gene was detected in 4% (139/3479) of molecularly confirmed NF1 index cases. Patients ranged between 4 months and 69 years old, with a median at 21 years old. A comprehensive clinical assessment showed that 93% (116/126) of *NF1*-deleted patients fulfilled the NIH criteria for NF1. More than half has café-au-lait spots, skinfold freckling, Lisch nodules, neurofibromas, neurological abnormalities, and cognitive impairment or learning disabilities. Comparison with previously described "classic" NF1 cohorts showed a significantly higher proportion of symptomatic spinal neurofibromas, dysmorphism, learning disabilities, malignancies, and skeletal and cardiovascular abnormalities in the *NF1*-deleted group. Maternal origin of the deletion was confirmed in 25 cases.

Conclusions: We described the largest *NF1*-deleted cohort to date and confirmed a more severe phenotype in *NF1*-deleted patients with a predominant maternal origin of the deletions.

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P04.061.B Identification of rare variants for nonsyndromic cleft lip with/without cleft palate in a cohort of multiplex families

Annika Scheer[1], *Fabian Brand*[2], *Julia Fazaal*[1], *Nina Ishorst*[1], *Peter M. Krawitz*[2], *Elisabeth Mangold*[1], *Kerstin U. Ludwig*[1]

[1]*Institute of Human Genetics, University of Bonn, School of Medicine & University Hospital Bonn, Bonn, Germany,* [2]*Institute for Genomic Statistics and Bioinformatics, University Hospital Bonn, Bonn, Germany.*

Nonsyndromic cleft with/without cleft palate (nsCL/P) are among the most common birth defects and have a multifactorial etiology. In the last years, genome wide association studies have identified around 40 common risk regions with small to moderate effect

sizes, but the contribution of rare variants with higher effect sizes has been studied to a lesser extent. The aim of our study was to systematically identify potential causal rare variants in 55 individuals from 10 multiplex families affected with nsCL/P. Each of the families had at least three affected family members over at least two generations. Whole genome sequencing was performed using Illumina Truseq Nano DNA Library Prep Kit, and variant calling was performed according to an in-house pipeline on a family-wise basis. Overall, 7,435,742 single-nucleotide variants and 45,627 structural variants were identified in the entire cohort, representing a unique resource for the analysis of rare events. In our first analysis we focused on the protein-coding regions and are currently applying technical, pedigree-based and frequency filtering in each of the families. In family 1, this analysis together with subsequent annotation in the Variant Effect Predictor identified 66 candidate variants in 65 genes, one of which has been previously suggested as risk gene in an independent exome sequencing study (*PLEKHA5*, Cox et al. 2018). Based on single-cell expression data during mouse embryonic development, additional candidate genes (e.g. *MPZL2, GPC6)* were also identified. The analysis of the remaining nine families is currently ongoing, and results will be presented at the conference.

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P04.062.C Analysis of single-cell based expression variability between candidate genes for syndromic and non-syndromic forms of orofacial clefting

Anna Siewert[1], *Julia Welzenbach*[1], *Benedikt Reiz*[2], *Elisabeth Mangold*[1], *Henning Dickten*[2], *Kerstin U. Ludwig*[1]

[1]*Institute of Human Genetics, University of Bonn, School of Medicine & University Hospital Bonn, Bonn, Germany,* [2]*FASTGenomics, Comma Soft AG, Bonn, Germany.*

Orofacial clefts (OFCs) are among the most common human birth defects. OFC can occur as an isolated phenotype, or as part of a more complex malformation syndrome. In the latter, additional tissues and organ systems show developmental differences beyond OFC. We here hypothesized that candidate genes involved in syndromic OFC-forms may be expressed in more cell types during embryonic development, compared to genes causing non-syndromic OFC. To study this, we re-analyzed recently published single-cell expression data from the Mouse Organogenesis Cell Atlas (Cao et. al 2019), covering the relevant time period for facial development from embryonic days E9.5-E13.5. We downloaded data from 100,000 sampled cells and performed data analyses using the R package Seurat (Stuart et. al 2019) and the analytical ecosystem FASTGenomics. Data was split by embryonic day to yield a developmental time frame. Candidate genes associated with non-syndromic OFCs were defined based on data from prior genome-wide association studies, while genes causing established OFC-syndromes (autosomal dominant (AD) and autosomal recessive) were retrieved from the literature. At each time point, we extracted the percentage of cell types expressing the respective gene and compared expression levels between the different groups. Our results show that on average, genes underlying AD forms of syndromic OFC are expressed in significantly more cell types during organogenesis compared to risk genes for non-syndromic OFC (P-values: $2.02x10^{-06}$ for E9.5; $1.44x10^{-04}$ (E10.5); $1.11x10^{-04}$ (E11.5); 0.002 (E12.5); 0.002 (E13.5)). This analysis will help to further understand the molecular pathways and etiological differences between syndromic and non-syndromic forms.

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P04.063.D Search for exonic homozygous/compound heterozygous variants in affected sib-pairs identifies novel candidate genes for nonsyndromic cleft palate

Nina Ishorst[1], *Helena Shkuro*[1], *Julia Fazaal*[1], *Ann Kathrin Hoebel*[1], *Holger Thiele*[2], *Teresa Kruse*[3], *Michael J. Dixon*[4], *Kerstin U. Ludwig*[1], *Elisabeth Mangold*[1]

[1]*Institute of Human Genetics, University of Bonn, School of Medicine & University Hospital Bonn, Bonn, Germany,* [2]*University of Cologne, Cologne Center for Genomics CCG, Cologne, Germany,* [3]*Department of Orthodontics, University of Cologne, Cologne, Germany,* [4]*Faculty of Biology, Medicine & Health, University of Manchester, Manchester, United Kingdom.*

Nonsyndromic cleft palate only (nsCPO) belongs to the typical forms of orofacial clefts and is a common congenital malformation. nsCPO is a multifactorial disorder with environmental and genetic factors contributing to disease risk. Of note, the genetic contribution is rather high with an estimated heritability of >90%. To date, one common risk locus for nsCPO is confirmed, and nine have been reported recently but still await independent replication. Epidemiological observations and genetic studies suggest that high penetrance rare/low frequency variants contribute to nsCPO, which might be inherited in an autosomal-recessive manner. In this study, we focussed on detection of novel nsCPO candidate genes by identifying compound heterozygous/homozygous variants. To that end, we reanalyzed whole-exome sequencing data from six affected sib-pairs born to unaffected parents. After filtering for population frequency (MAF < 5%) and manual inspection of reads, we detected 169 putative compound heterozygous and 13 putative homozygous variants. We next prioritized these 182 variants/80 candidate genes based on (1) the functional effects at transcriptional level and *in silico* predictions, (2) intolerance for a certain class of rare variation, and (3) expression in mouse embryonic palatal shelves at embryonic day E13.5. This resulted in a list of the 32 most promising candidate genes, which include one compound heterozygous situation within *GRHL3*, which has been implicated in nsCPO, and novel genes encoding proteins involved in cell migratory processes, such as DDR2, a binding partner of the gene product of the well established clefting gene *CDH1*.

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P04.064.A Systematic analysis of non-coding *de novo* mutations from whole genome sequence data of triads with non-syndromic cleft lip with/without cleft palate

Hanna K. Zieger[1], *Leonie Weinhold*[2], *Axel Schmidt*[1], *Manuel Holtgrewe*[3], *Stefan A. Juranek*[4], *Anna Siewert*[1], *Frederic Thieme*[1], *Fabian Brand*[5], *Julia Welzenbach*[1], *Dieter Beule*[3,6], *Katrin Paeschke*[4], *Peter M. Krawitz*[2], *Kerstin U. Ludwig*[1]

[1]*Institute of Human Genetics, University of Bonn, School of Medicine & University Hospital Bonn, Bonn, Germany,* [2]*Institute for Medical Biometry, Informatics and Epidemiology, University Hospital Bonn, Bonn, Germany,* [3]*Core Unit Bioinformatics, Berlin Institute of Health, Berlin, Germany,* [4]*Department of Oncology, Hematology and Rheumatology, University Hospital Bonn, Bonn, Germany,* [5]*Institute*

for Genomic Statistics and Bioinformatics, University Hospital Bonn, Bonn, Germany, [6]Max Delbrück Center for Molecular Medicine, Berlin, Germany.

Non-syndromic cleft lip with/without cleft palate (nsCL/P) is a common multifactorial disorder with strong genetic contribution. Here, we systematically investigate the contribution of *de novo* mutations (DNMs) to nsCL/P risk, using whole-genome sequence (WGS) data for 211 nsCL/P and 284 non-cleft reference trios from the Kids First Project. For the total set of 31,490 DNMs, overall comparison between cohorts did not show conclusive differences, neither in absolute numbers nor when weighted for different functional scores. However, we observed nominally significant accumulation of non-coding DNMs at bivalent TSS/enhancer chromatin states in nsCL/P during human embryonic face development at Carnegie Stage 15 ($p = 0.0269$), and a nominally significant enrichment of non-coding DNMs in topologically associating domains at two GWAS risk loci, i.e. 4q28.1 (7 cases, 0 controls, $p = 0.0008$) and $2p21_{PKDCC}$ (7 cases, 2 controls, $p = 0.0161$). We finally used transcription factor (TF) binding information to identify TFs with potential key role in nsCL/P etiology. Based on position weight matrices, we predicted TF binding sites for 810 human TFs, and calculated changes of binding capacity at 28,773 DNM-sites. We observed a significant enrichment of DNM-hits for motif *TFAP2A* in nsCL/P, and identified *ATF3*, *MSC* and *HES5/7* as potential TF candidates. Notably, for *MSC* and *ATF3*, this finding was supported by a strong quantitative effect on the predicted binding change, which for *MSC* is currently validated using in vitro assays. Our study provides novel insights into nsCL/P etiology and suggests a TF-based approach that can be used to annotate non-coding risk variants from WGS data.

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P04.065.B Chondrocyte protein co-expression network analysis reveals a link between ECM mechanosensing and glucose metabolism in osteoarthritis

Aspasia Destouni[1], *Konstantinos C. Tsolis*[2], *Anastassios Economou*[2], *Ioanna Papathanassiou*[3], *Charalambos Balis*[1], *Evanthia Mourmoura*[1], *Aspasia Tsezou*[1,3]

[1]*Laboratory of Cytogenetics and Molecular Genetics, Faculty of Medicine, University of Thessaly, Larissa, Greece,* [2]*Department of Microbiology and Immunology, Rega Institute for Medical Research, KULeuven, Leuven, Belgium,* [3]*Department of Biology, Faculty of Medicine, University of Thessaly, Larissa, Greece.*

Introduction: Knee osteoarthritis (OA) is the second most common structural OA disorder affecting approximately 22.9% of individuals 40 years and over globally. The only currently available effective treatment is total knee joint replacement, which is performed at the late stages of the disease. The lack of effective disease-modifying drugs and preventive tools highlights our incomplete understanding of the fundamental biological aspects of osteoarthritis.

Materials and methods: We performed label-free shotgun LC-MS in primary chondrocytes obtained from the articular cartilage of 10 OA and 6 healthy individuals. We implemented the statistical concepts of Weighted Gene Co-expression Network Analysis to reconstruct in an unbiased way the organisation of the chondrocyte proteome and to parse the shared chondrocyte protein interactome in disease associated modules.

Results: Chondrocyte proteome is parsed into functional modules with well characterized significance to the disease, such as core structural components of the cartilage ECM which form a meta-module with proteins mediating glycolysis. Meta-module protein abundance is reduced whilst proteins mediating focal adhesion and cytoskeletal dynamics are increased in OA. *COL11A1* and *TNC* have significant associations with OA in the GWAS catalog and are members of the ECM module indicating that variants could be exerting a detrimental effect in the context of ECM mechanosensing-based regulation of chondrocyte metabolism.

Conclusions: Our systems analysis recapitulates hallmarks of OA and offers new insights into the modular structure of the protein interactome that is associated with OA chondrocyte biology. **Grant:** iStemTheOS, Grant No. MIS 5033630/ELKE5876 from the Hellenic Foundation for Research & Innovation.

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P04.066.C A family with Osteochondritis Dissecans and multiple fractures harboring *COL9A2* and *PLS3* Mutations

Estelle Arnaud Gouttenoire[1], *Sophie Lang-Andrey*[2], *Françoise Lemaire-Girard*[1], *Jean Dudler*[2], *Michael Morris*[1]

[1]*SYNLAB Switzerland, Department of Genetics, Lausanne, Switzerland,* [2]*HFR Fribourg, Department of Rheumatology, Fribourg, Switzerland.*

A 25-year-old woman consulted in our specialized genetic rheumatology clinic, with symptomatology evolving since the age of 11, with knee pain and episodes of joint blockage, MRI positive axial spondyloarthropathy and hyperlaxity. The diagnosis of bilateral osteochondritis dissecans (OCD) was made at the age of 22. She later developed ankle pain also related to OCD and spiral fracture of the tibia and proximal fibula, without trauma. The proband's brother had multiple fractures and joint blockage since childhood, and 1 maternal uncle had joint blockage since childhood. The proband's mother had pain in childhood and has been suffering from pain in her arms, knees and ankles for 1 year. The maternal grandmother has lumbar disc degeneration and hearing problem. NGS of a 251-gene skeletal dysplasia panel revealed that proband is double-heterozygote for *COL9A2* c.186G>A and *PLS3* c.827G>A, p.(Trp276*). *PLS3* mutations cause X-linked osteoporosis with fractures. *COL9A2* mutations have been associated with AD multiple epiphyseal dysplasia, characterized by early onset of pain associated with OCD in some patients, and also with intervertebral disc disease. Interestingly, these 2 variants segregate in the family. This approach confirms the importance of genetic consultation in familial rheumatological conditions and reveled the existence of 2 overlapping genetic connective tissue pathologies.

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P04.067.D Atypical type VI Osteogenesis Imperfecta mouse models the intersection of *IFITM5* and *SERPINF1* pathways in patients

Gali Guterman Ram[1], *Ghazal Hedjazi*[2], *Chris Stephan*[3], *Stéphane Blouin*[2], *Victoria Schemenz*[4], *Wolfgang Wagermaier*[4], *Jochen Zwerina*[2], *Peter Fratzl*[4], *Kenneth M. Kozloff*[3], *Nadja Fratzl-Zelman*[2], *Joan C. Marini*[1]

[1]Section on Heritable Disorders of Bone and Extracellular Matrix, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA, [2]Ludwig Boltzmann Institute of Osteology at the Hanusch Hospital of OEGK and AUVA Trauma Centre Meidling, 1st Medical Department, Hanusch Hospital, Vienna, Austria, [3]Department of Orthopaedic Surgery, University of Michigan, Ann Arbor, MI, USA, [4]Max Planck Institute of Colloids and Interfaces, Dept. of Biomaterials, Potsdam, Germany.

Osteogenesis Imperfecta (OI) is a well-known skeletal dysplasia. Type V OI, caused by recurrent dominant mutation in *IFITM5*/BRIL, and type VI OI, caused by recessive null mutations in *SERPINF1*/PEDF, have distinct features. *IFITM5*-S40L mutations causes severe dominant atypical type-VI OI (aVI) with phenotype, bone histology and decreased cellular secretion of PEDF similar to type VI OI.

Our objective is understanding the pathways connecting *IFITM5* and *SERPINF1* in bone development.

We generated an *Ifitm5* S42L knock-in mouse model. Newborn *Ifitm5* S42L mice, heterozygous(HET) and homozygous(HMZ), are non-lethal, have flared rib cage, shoulder and knee dislocations. Radiographically, ≈50% HET mice exhibit fractures and 96% of HMZ incur fractures at multiple ages. Similar to patients, heterozygous males have normal PEDF level and increased serum ALP ($p < 0.01$). Mechanical testing of 2-month HET and HMZ showed reduced stiffness, yield and fracture load, with markedly increased brittleness. On μCT, HET mice have decreased Ct.Th, increased BV/TV, Tb.N. Whole-body DXA-aBMD was significantly decreased, with HMZ are more severe than HET($p < 0.01$). qBEI of cortical bone revealed hypermineralization in 1-and 2-month HET, with increased CaMean, CaPeak($p < 0.05$) and CaHigh($p < 0.0001$, $p = 0.0027$ respectively) and increased density and decreased area (both $p < 0.001$) of osteocyte lacunar sections. Second harmonic generation florescence microscopy revealed HET bones contain mostly disordered matrix($p < 0.0001$). Cultured calvarial and long bone osteoblasts exhibit differences in differentiation pattern, dependent on mating scheme, age and skeletal site.

Our murine model with physiologic levels of *Ifitm5* S42L expression recapitulates patient phenotype and will be used to investigate mechanisms and pathways involving *Ifitm5* and *Serpinf1*.

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P04.068.A Study of OI patient osteoblasts to investigate phenotypic variability of dominant osteogenesis imperfecta

Milena Jovanovic, *Apratim Mitra, Ryan Dale, Joan Marini*

NICHD/NIH, Bethesda, MD, USA.

Osteogenesis Imperfecta (OI) is a heterogeneous bone disorder characterized by bone fractures, growth deficiency and skeletal defects. An important and unexplained feature of OI and many dominant disorders is phenotypic variability with the same mutation. We present the first comparative study of osteoblast differentiation from normal pediatric controls vs OI patients with phenotypic variability. We focused on *COL1A1* mutations Gly352-Ser and Gly589Ser. For each mutation we investigated osteoblasts in two unrelated patients, one with mild and one with severe phenotype. OB cell layer and secreted collagen were overmodified in all patients, shown by ^{3}H steady-state assay, indicating delayed folding; however, quantitative analysis of hydroxylysines did not correlate with patient severity. Alizarin Red staining showed patient OB deposit significantly less mineral in vitro than controls ($p < 0.05$), with OB from severe patients depositing significantly less mineral than from mild patients ($p < 0.05$). RNA-Seq transcriptomics of differentiated osteoblasts showed proteasomal protein degradation, autophagy, and vesicle organization pathways upregulation vs controls, while protein translation was downregulated. OB from both severe patients have upregulation of pathways related to ubiquination vs controls. RNA-Seq is currently being validated by RT-qPCR and western blot and the functional significance of pathways investigated. This study will lead to novel insights into OI osteoblast differentiation, the respective roles of OB and matrix in phenotypic variability and the effect of substitution position along the collagen helix.

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P04.069.B Bone cell functions in *PPIB* knockout mouse model for type IX osteogenesis imperfecta are distinct from classical dominant OI

Ying Liu[1], *Theresa Hefferan*[2], *Nadja Fratzl-Zelman*[3], *Joan Marini*[1]

[1]*Section on Heritable Disorders of Bone and Extracellular Matrix, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA,* [2]*Biomaterials and Histomorphometry Core Laboratory, Mayo Clinic, Rochester, MN, USA,* [3]*Ludwig Boltzmann Institute of Osteology at the Hanusch Hospital of OEGK and AUVA Trauma Centre Meidling, 1st Medical Department, Hanusch Hospital, Vienna, Austria.*

Osteogenesis imperfecta (OI) is a collagen-related bone disorder, which is caused by either dominant mutations in collagen, or recessive defects in genes encoding collagen-interacting proteins. Cyclophilin B (CyPB), encoded by *Ppib*, functions as a procollagen prolyl 3-hydroxylation complex component (P3H1/CRTAP/CyPB) and independently as the major peptidyl-prolyl cis-trans isomerase (PPIase) catalyzing collagen folding. Mutations in *Ppib* cause recessive type IX OI. We reported previously that *Ppib*$^{-/-}$ mice have abnormal type I collagen post-translational modification and crosslinks. This study focuses on *Ppib*$^{-/-}$ bone cell functions, utilizing histomorphometry and qBEI analysis of femoral tissue, RT-PCR and alizarin red staining of osteoblasts, and osteoclast differentiation. Histomorphometry of *Ppib*$^{-/-}$ femora reveals reduced trabecular thickness ($p < 0.05$), trabecular number ($p < 0.01$), bone volume ($p < 0.001$), and cortical thickness ($p < 0.0001$). Distinct from high turnover in dominant OI, *Ppib*$^{-/-}$ osteoblast number and surface are significantly decreased ($p < 0.01$; $p < 0.05$). Osteoclast number does not differ between KO and WT bone, as well as in vitro osteoclast differentiation. *Ppib*$^{-/-}$ femora show reduced osteoid volume ($p < 0.05$), MAR ($p < 0.0001$) and BFR/BS ($p < 0.01$) vs WT. *Ppib*$^{-/-}$ osteoblasts reveal elevated matrix mineralization ($p < 0.001$), consistent with increased expression of late osteoblast differentiation markers *Sost, Mepe, Phex, Dmp1*, vs WT. qBEI analysis yields increased CaMean, CaPeak (both $p < 0.001$) and CaHigh values ($p = 0.014$) in femoral midshaft cortical bone of KO vs WT. Cyclophilin B/*Ppib* KO mice, modelling type IX OI, have bone cell functions distinct from classical dominant OI. PPIB KO bone has a low turnover cellular pattern with decreased osteoblast number and bone formation, increased mineralization, and normal osteoclast numbers.

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P04.070.C Genome sequencing discloses a homozygous noncoding deletion of 72kb upstream of *SNX10* in autosomal recessive osteopetrosis

Gandham SL Bhavani[1], *Prajna Udupa*[1], *Neethukrishna Kausthubham*[1], *Sandip Bartakke*[2], *Katta M. Girisha*[1]

[1]*Department of Medical genetics, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal, India,* [2]*Department of Clinical Hematology, Aditya Birla Memorial Hospital, Pune, India.*

Introduction: Autosomal recessive osteopetrosis (ARO) is a rare genetic disorder of bone resorption caused by defective osteoclasts resulting in increased bone density. Other characteristic features of this condition are macrocephaly, visual impairment, splenomegaly, and bone marrow failure. The incidence of this disorder is 1 in every 250,000 live births with onset ranging from neonatal stage to adulthood. Eight genes are associated with autosomal recessive osteopetrosis. Pathogenic variants in *SNX10* (sorting nexin 10) contribute to ARO in 4% of cases. SNX10 helps in osteoclast differentiation by RANKL-stimulation.

Materials and methods: We ascertained a four-year old boy born to consanguineously married couple. Detailed clinical evaluation and skeletal survey were done. We performed exome sequencing followed by genome sequencing to identify the genetic etiology.

Results: Proband exhibited macrocephaly, visual impairment, pectus carinatum and hepatosplenomegaly. Increased bone density, sandwich vertebrae and bone-in-bone appearance were observed on radiographs. Exome sequencing was non-diagnostic. Whole genome sequencing identified a large homozygous indel, g.26263639_26335652delinsCAA, which includes ~72kb deletion along with three-nucleotides insertion in the *SNX10* gene. This deletion encompasses noncoding exon 1, 5′ untranslated region, the promoter region, and upstream of SNX10. Parents are carriers of this deletion.

Conclusion: The novel ~72kb indel in noncoding region of *SNX10* is the likely cause of autosomal recessive osteopetrosis in our patient. We hereby report the second large homozygous indel in *SNX10* resulting in autosomal recessive osteopetrosis.

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P04.071.D The use of bisphosphonates to treat osteoporosis in patients with Lysinuric Protein Intolerance

Jos Draaisma, *Joyce Geelen, Maaike de Vries, Anne Dittrich*

Radboudumc Amalia children's hospital, Nijmegen, Netherlands.

Background: Lysinuric Protein Intolerance (LPI) is an autosomal metabolic disorder. Patients present with failure to thrive, cytopenia, acute encephalopathy or developmental disability. Long term complications includes also low bone mineral density. In general the treatment is focused on the prevention of hyperammonemia. The aim of this report is to propose the use of bisphosphonates for osteoporosis in patients with LPI.

Methods: Clinical description of a patient and review of literature.

Results: A 8-year old girl was born to non-consanguineous parents. She had a normal course until the age of 6 years. Since than she had multiple fractures including multiple vertebral fractures at different occasions due to mild trauma. Further investigation led to the genetically confirmed diagnosis LPI. The lumbar Z-score was -3.7. She was treated with intravenous pamidronate and supplemental calcium and vitamin D. No further fractures occurred. After one year the z-score increased to -1.9, after two year -1.3.

Discussion: In a cohort study performed in France 80% of the patients with LPI was diagnosed with osteopenia. In a series of 29 patients in Finland, 69% of patients had one or more fractures, mostly in childhood. The exact mechanism of the osteoporosis in LPI is still not fully understood. The normal initial therapy of patients with LPI (a protein-restricted diet and supplemental L-citrulline) does not change the signs of low bone mineral density. Bisphosphonates can be used to treat osteoporosis in patients with LPI.

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P04.072.A Severe osteosclerosis and early poor outcome in a patient with TCIRG1 mutation

Elena Sukarova-Angelovska, *Marina Krstevska-Konstantinova, Natasa Alulovska, Svetlana Kocheva*

Pediatric Clinic, Skopje, Macedonia, The Former Yugoslav Republic of.

Osteopetrosis is a heterogeneous group of disorders of bone metabolism triggered by defective osteoclast function. Increased bone density leads to worsening of many body functions. Among all known genetic causes, loss of function mutation in TCIRG1 gene is responsible for the disease in 70% of the cases. Impaired function of α3 subunit V-ATPase leads to inappropriate extracellular acidification, osteoclast proliferation and unossified osteoid matrix. We present a male neonate that admitted our clinic shortly after birth due to the suspicion of sepsis. Episodes of multifocal osteomyelitis occurred successively. He had several dysmorphic features-macrocephaly, protruded forehead, prominent eyes, receding mandible, large ears. Several painful swellings were present due to the multiple bone fissures. X-ray series confirm sclerosis of all bones, appearance of dense bone formation, sandwich vertebrae, widened growth plate and calvarial bone thickening. The mutation of TCIRG1 was found (G 2415A). Development of side effects occurred within the first several months (metabolic acidosis, hepatosplenomegalia, anemia, skeletal deformities; blindness and deafness). He developed hydrocephalus and consecutive seizures at 9 months and died at the age of 11 months due to respiratory failure. Although the mutations in TCIRG1 gene are frequent cause of AR osteopetrosis, there is a wide heterogeneity of clinical presentation - from moderate to malignant, primarily due to the different mutation and consecutive acidification error. Genotype-phenotype studies are limited; moreover, the same mutation could give rise to different phenotypes. Further investigation is needed in order to elucidate all contributing mechanisms that can indicate the severity of the disease.

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P04.074.C Periodontal (formerly type VIII) Ehlers-Danlos syndrome: description of 12 novel cases and expansion of the clinical phenotype

Salima EL CHEHADEH[1,2], *Anne LEGRAND*[3,4], *Corinne STOETZEL*[2], *Véronique GEOFFROY*[2], *Clarisse BILLON*[3,4], *Salma ADHAM*[4], *Xavier JEUNEMAITRE*[3,4], *Roland JAUSSAUD*[5], *Jean Muller*[2,6], *Elise SCHAEFER*[1], *Karelle BENISTAN*[7], *Sébastien GAERTNER*[8,9], *Agnès BLOCH-ZUPAN*[10,11,12], *Marie-Cécile MANIERE*[10,11,12], *Catherine PETIT*[10,11,13], *Anne-Claire Bursztejn*[14,15], *Laurence BAL*[16], *Anthony REYRE*[17], *Tiffany BUSA*[18], *Hélène DOLLFUS*[1,2], *Dan LIPSKER*[14,19]

[1]*Service de Génétique Médicale, Institut de Génétique Médicale d'Alsace (IGMA), Hôpitaux Universitaires de Strasbourg, Hôpital de Hautepierre, Strasbourg, France,* [2]*Laboratoire de Génétique Médicale, UMRS_1112,*

Institut de Génétique Médicale d'Alsace (IGMA), Université de Strasbourg et INSERM, Strasbourg, France, [3]Université de Paris, INSERM, U970, Paris Centre de Recherche Cardiovasculaire, Paris, France, [4]Centre de Référence des Maladies Vasculaires Rares, AP-HP, Hôpital européen Georges Pompidou, Paris, France, [5]Département de médecine interne immunologie clinique, CHRU de Nancy et université de Lorraine, Nancy, France, [6]Laboratoire de diagnostic génétique, Nouvel hôpital civil, Hôpitaux Universitaires de, Strasbourg, France, [7]Centre de référence des syndromes d'Ehlers-Danlos non vasculaires, Hôpital Raymond Poincaré, Garches, Assistance Publique Hôpitaux de Paris, Garches, France, [8]Service des Maladies Vasculaires - Hypertension Artérielle, Hôpitaux Universitaires de Strasbourg, Strasbourg, France, [9]INSERM, UMR 1260, Regenerative Nanomedicine, FMTS, Strasbourg, France, [10]Université de Strasbourg, Faculté de Chirurgie Dentaire, Strasbourg, France, [11]Hôpitaux Universitaires de Strasbourg, Pôle de Médecine et Chirurgie Bucco-Dentaires, Hôpital Civil, Centre de référence des maladies rares orales et dentaires, O-Rares, Filière Santé Maladies rares TETE COU, European Reference Network ERN CRANIO, Strasbourg, France, [12]Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), INSERM U1258, CNRS-UMR7104, Université de Strasbourg, Illkirch-Graffenstaden, France, [13]INSERM, UMR 1260 'Osteoarticular and Dental Regenerative Nanomedicine', Faculté de Médecine, Fédération de Médecine Translationnelle de Strasbourg (FMTS), Strasbourg, France, [14]Clinique Dermatologique, Hôpitaux Universitaires de Strasbourg, Strasbourg, France, [15]Service de dermatologie, hôpitaux de Brabois, CHU de Nancy, Vandœuvre-lès-Nancy, France, [16]Timone Aortic Center, Timone Hospital, APHM ; Department of Vascular Surgery, Timone Hospital, APHM, Marseille, France, [17]Service de neuroradiologie interventionnelle, CHU de Marseille, Hôpital La Timone, Marseille, France, [18]Département de génétique médicale, CHU de Marseille, Hôpital La Timone, Marseille, France, [19]Faculté de Médecine, Université de Strasbourg, Strasbourg, France.

Periodontal Ehlers-Danlos syndrome (pEDS) is a rare condition caused by autosomal dominant pathogenic variants in the *C1R* and *C1S* genes, encoding subunits C1R and C1S of the first component of the classical complement pathway. It is predominantly characterized by early-onset severe periodontitis with premature tooth loss, pretibial hyperpigmentation and skin fragility. Rare arterial complications have been reported, but venous insufficiency is rarely described. Here we report twelve novel patients carrying either *de novo* (4/12) or inherited (8/12) heterozygous pathogenic variants in *C1R* and *C1S*, in order to characterize their clinical phenotype, with a focus on the vascular features. All presented with typical pEDS clinical signs including severe periodontitis (except the youngest patient aged 3 years) with complete tooth loss in three patients before the age of 30. Three patients and one relative also displayed widespread venous insufficiency leading to chronic varicose leg ulcers. One patient suffered an intracranial aneurysm with vascular complications in 3 relatives including thoracic and abdominal aortic aneurism and dissection and intracranial aneurysm rupture. This work highlights the importance of early diagnosis of pEDS to direct appropriate dental care and precise the genetic counselling. It also confirms that vascular complications are possible, although they are not frequent, which leads us to propose to carry out a first complete vascular evaluation after the diagnosis. Larger case series are however needed to precise the frequency of these vascular complications and to improve our understanding of the link between complement pathway activation and connective tissue alterations observed in these patients.

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P04.075.D Comprehensive analysis of the genetic determinants of proportionate short stature by targeted NGS

Angel Campos-Barros[1,2], *Elena Vallespín*[1,2], *Karen E. Heath*[1,2], *Angela Del Pozo*[1,2], *Mario Solís*[1], *Isabel González-Casado*[3], *Paula Casano*[4], *Isabel R. Martínez-Orga*[1], *María Fernández-Elvira*[1], *Victoria E. Fernández-Montaño*[1]

[1]*INGEMM, IdiPAZ, Hospital Universitario La Paz, Madrid, Spain,* [2]*CIBER de Enfermedades Raras (U753), ISCIII, Madrid, Spain,* [3]*Dept. of Pediatric Endocrinology, Hospital Universitario La Paz, Madrid, Spain,* [4]*Dept. of Pediatric Endocrinology, Hospital Sant Joan de Déu, Barcelona, Spain.*

Proportionate short stature is a common condition (3% of general population) with a heterogeneous genetic etiology. Despite the advances in the understanding of pathophysiological growth regulation, the underlying genetic causes are still underdiagnosed. *Aim and subjects:* Molecular genetic screening of a cohort of 201 pediatric cases (86 females and 115 males) with proportionate short stature (height< -2.0 SDS) by targeted NGS.

Methods: Genomic DNA samples were analyzed with a custom panel (Seq-Cap-EZ; Nimblegen, Roche) including 331 known genes implicated in the etiology of syndromic and non-syndromic proportionate short stature. Variant prioritization was based on sequence quality assessment (Q>30); coverage (mean >90x; %bp>20x >80%); population frequency (MAF <1% in gnomAD controls), variant effect (missense, nonsense, frameshift, splicing effect) and *in silico* pathogenicity prediction (CADD_1.4 score >20).

Results and discussion: 97 potentially deleterious variants were identified in a total of 84 (41.8%) index cases, of which, 35 (57.3%) in genes involved in GH-IGF1 signaling (i.e. *GHRH, GHRHR, GHR, IGF1R, PTPN11, IGF2R*), and 26 (26.8%) in genes regulating IGF1 bioavailability (*IGF1, IGFALS, IGFBP4, PAPPA2, PAPPA*). The remaining variants were in genes: i) associated with syndromic forms of proportional short stature, *KDM6A* (Kabuki-syndrome; n = 3), *NFKB2* (DAVID syndrome, n = 3), *CUL7* (3M-syndrome, n = 3), and *BTK* (n = 1); ii) implicated in pituitary morphogenesis, *PROKR2* (n = 5), *IHH* (n = 5); iii) extracellular matrix genes, *ACAN* (n = 11); or iiii) in genes encoding paracrine factors of the growth plate, *NPPC*; (n = 2) and *NPR2* (n = 3). Targeted NGS analysis significantly improves the diagnostic rate of proportionate short stature. Grants PI 12/00649; 18/00402 (ISCIII); ENDOSCREEN S2010/BMD-2396

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P04.076.A Plasma inorganic pyrophosphate levels correlate to specific phenotypes and variant archetypes of *ABCC6* in pseudoxanthoma elasticum patients

Matthias Van Gils[1,2], *Justin Depauw*[1], *Shana Verschuere*[1,2], *Lukas Nollet*[1,2], *Olivier M. Vanakker*[1,2]

[1]*Center for Medical Genetics Ghent, Ghent, Belgium,* [2]*Department of Biomolecular Medicine, Ghent University, Ghent, Belgium.*

Introduction: Pseudoxanthoma elasticum (PXE) is an ectopic mineralization disease caused by biallelic *ABCC6* mutations. Decreased inorganic pyrophosphate (PPi) levels have been linked to ectopic mineralization in patients. However, whether correlations exist between PPi levels and the phenotype or *ABCC6* genotype is unknown.

Methods: PPi levels of 128 blood samples (92 [62 patients], 22 [21 carriers] and 14 [14 controls]) were measured. *ABCC6* pathogenic variants (C3-C5) were considered. PXE phenotypes were assessed using the Phenodex scoring (PS) system at initial patient sampling. Two-fold statistical analysis was performed: bulk analysis and single/first sample analysis. Correlations between PPi, age, sex and PS were investigated. Based on mutation archetypes (erroneous mRNA [D], nonsense [N] and missense [M] variants) and increasingly stringent variant selection genotype-PPi correlations were assessed.

Results: Patients and carriers had lowered PPi levels (respectively ±51% and ±78% of controls; $P < 0.001$) with sample range overlap between all groups. Sex (Bulk: $P = 0.010$; Single: $P = 0.060$) and age (Bulk: $P = 0.005$, $r = 0.288$, Single: $P = 0.015$, $r = 307$) significantly affected PPi levels only in patients. A significant inverse correlation between PPi levels and cardiac PS scores was identified ($P = 0.041$, $r = -0.263$) alongside a weak inverse association between PPi and vascular PS ($P = 0.09$). Significant differences in PPi levels between D+M and N+M genotypes were found in bulk analysis only ($P < 0.05$).

Conclusions: PPi is reduced in patients and carriers but overlap between groups suggests other pro-mineralization factors are also relevant in at least some patients. Correlations between PPi and cardio(vascular) phenotypes suggest PPi has promise as a biomarker; genotype-correlations however require further confirmation.

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P04.077.B lncRNA gene variants SPRR2C rs2291979 and LOC105375120 rs4724102 are associated with psoriasis

Laimutis Kucinskas[1], *Migle Anilionyte*[1], *Vesta Kucinskiene*[2], *Skaidra Valiukeviciene*[2]

[1]*Institute of Biological System and Genetic Research, LUHS, Kaunas, Lithuania,* [2]*Department of Skin and Venereal Diseases, Lithuanian University of Health Sciences (LUHS), Kaunas, Lithuania.*

The aim: *to determine the significance of SNV of genes SPRR2C rs2291979, LOC105375120 rs4724102 and LINC01698 rs75193730 in the genotypes of psoriasis and control group persons and evaluate associations between genotype and clinical signs of psoriasis*. **Patients and Methods**. *Ninety five samples from psoriasis patients' group and 77 samples from a control group of healthy people were examined. Genomic DNA was extracted from peripheral blood leukocytes by DNA salting out procedure. Genotyping was performed by real time polymerase chain reaction and data were analysed by statistical analysis program IBM SPSS statistics*.

Results: *SPRR2C SNV rs2291979 frequency of A allele was 7,9 % compare to 1,3 % of control group ($p = 0,011$). LOC105375120 genotype G/G frequncy was 57,9 % in patient group compare with 28,6 % control group ($p < 0,001$) and allele G frequency was 77,4 % in patient group ($p < 0,00001$). No statistically significant difference was found between LINC01698 SNV rs75193730 genotypes and alleles.*

Conclusions: *Statisticaly significant higer frequency was determinated of SNV SPRR2C rs2291979 A allele and LOC105375120 G/G genotype and G allele compare with control group. No statistically significant associations of clinical signs and alleles and genotypes of genes SNV SPRR2C rs2291979, LOC105375120 rs4724102 and LINC01698 rs75193730 were determinated*.

L. Kucinskas: None. **M. Anilionyte:** None. **V. Kucinskiene:** None. **S. Valiukeviciene:** None.

P04.078.C The HRAS-RIN1 signaling axis controls integrin trafficking in keratinocytes and its dysregulation contributes to the epidermal manifestation in Costello Syndrome

Theresa Nauth, *Laura Isabel Brandenstein, Verena Rickassel, Georg Rosenberger*

University Medical Center Hamburg-Eppendorf, Institute of Human Genetics, Hamburg, Germany.

Germline missense mutations in the HRAS gene cause Costello syndrome (CS), a rare developmental disorder characterized by a typical facial gestalt, postnatal growth deficiency, intellectual disability, predisposition to malignancies as well as skeletal, cardiac and dermatological abnormalities. The molecular pathophysiology caused by heterozygous HRAS gain-of-function mutations have been analyzed in various tissues and cell types. However, up to date the molecular basis for cutaneous manifestations in CS is largely unknown. To study epidermal pathobiology, we generated permanent human keratinocyte cells (HaCaT) stably expressing wild type $HRAS^{WT}$ or CS-associated $HRAS^{Gly12Ser}$ and screened for keratinocyte-specific HRAS binding partners by affinity purification and quantitative mass spectrometry. We identified and verified RIN1 (Ras and Rab interactor 1) as most important interaction partner of active HRAS variants in keratinocytes. By its dual function as an activator of ABL1/2 and RAB5A, RIN1 is involved in cytoskeletal remodeling and endosomal sorting of cell surface receptors, such as integrins. FACS-based integrin endocytosis assays showed aberrant integrin trafficking in keratinocytes expressing $HRAS^{Gly12Ser}$ and application of primaquine revealed integrin recycling to be essentially affected. By immunocytochemistry, we detected an increase of intracellular vesicular β1 integrin which strongly co-localized with RAB5, RAB21 and EEA1. The altered bioavailability of integrins in keratinocytes expressing $HRAS^{Gly12Ser}$ was associated with impaired cell spreading. Our data demonstrate that CS-associated $HRAS^{Gly12Ser}$ interferes with RIN1-RAB5 mediated endosomal sorting of integrins and, thus, adhesion-dependent processes. We conclude that dysregulation of receptor trafficking and cell adhesion are relevant in the pathobiology of CS.

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P04.079.D IRAK2 is associated with rheumatoid arthritis susceptibility

Rim Sghiri[1], *Hana BenHassine*[2], *Asma Boumiza*[2], *Khadija Baccouche*[3], *Foued Slama*[2], *Adel Almogren*[1], *Zahid Shakoor*[1], *Elyes Bouajina*[3], *Ramzi Zemni*[2]

[1]*King Saud University, Riyadh, Saudi Arabia,* [2]*Faculte de Medecine de Sousse, Sousse, Tunisia,* [3]*Hopital Farhat Hached de Sousse, Sousse, Tunisia.*

Objectives: Investigate the association of the single nucleotide polymorphisms of interleukin-1 receptor-associated kinase 2 (IRAK2) rs3844283 and rs708035 with rheumatoid arthritis (RA).

Patients and methods: IRAK2 rs3844283 and rs708035 genotyping was determined by mutagenically separated PCR in a cohort of 222 (30 men, 192 women, mean age 49 years) adult RA patients and 224 matched controls.

Results: IRAK2 rs3844283 C allele was detected in 66% of RA patients and 74% of controls. The CC genotype was the most frequent genotype in both RA patients (45.5%) and the controls (56.3%). The G allele was found to be associated with RA susceptibility ($OR = 1.47$, 95% $CI = 1.10\text{-}1.96$, $p = 0.008$). The GG genotype was found to be associated with RA in the co-dominant and the dominant models ($OR = 2.03$, 95% $CI = 1.08\text{-}3.81$, $p = 0.042$ and $OR = 1.54$, 95% $CI = 1.06\text{-}2.23$, $p = 0.023$, respectively). IRAK2 rs708035 was found not to be in the Hardy-Weinberg

equilibrium. The hyperfunctional IRAK2 rs708035 A allele was more frequent in RA patients than in controls (69.9 versus 62.2%, respectively, $p = 0.015$). Moreover, IRAK2 rs708035 and IRAK2 rs3844283 were in linkage disequilibrium and the GA haplotype was significantly more frequent in RA patients than in controls ($p = 0.034$).

Conclusion: This study for the first time ever reports the association of IRAK2 rs3844283, IRAK2 rs708035, and the corresponding haplotypes with RA. Functional studies are recommended to elucidate the risk posed by the GA haplotype for the development of RA.

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P04.080.A A TRAF6 genetic variant is associated with low bone mineral density in rheumatoid arthritis

*Rim Sghiri[1], **Hana BenHassine**[2], Najla El Amri[3], Khadija Baccouche[3], Foued Slama[2], Zahid Shakoor[4], Adel Almogren[4], Elyes Bouajina[3], Ramzi Zemni[2]*

[1]king Saud University, Riyadh, Saudi Arabia, [2]Faculte de Medecine de Sousse, Sousse, Tunisia, [3]Hopital Farhat Hached de Sousse, Sousse, Tunisia, [4]King Saud University, Riyadh, Saudi Arabia.

Objectives: This study was aimed at investigating the association of the single nucleotide polymorphism of tumor necrosis factor receptor associated factor 6 (TRAF6), rs540386, with low bone mineral density (BMD) among patients with rheumatoid arthritis (RA).

Patients and Methods: TRAF6 rs540386 genotyping was performed by mutagenically separated PCR in a cohort of 188 (23 men, 165 women, median age, 56.2 years) adult RA patients and 224 age and gender-matched controls. BMD was measured using dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy advance scans, GE Healthcare, USA).

Results: Among the RA patients, 64 (55 women, 9 men) had low BMD comprising of 57 patients with osteoporosis and 7 with osteopenia. Whereas TRAF6 rs540386 was not associated with RA susceptibility, it was however found to be a risk factor for reduced lumbar spine Z-score in the recessive model (OR = 3.34, 95% CI = (1.01-11.00), $p = 0.038$). This association was confirmed further in the multivariate logistic regression analysis taking into account several potential confounding factors (OR = 3.34 (1.01-11.00), $p = 0.048$). In addition, mean total femur Z-score was found to be reduced in TT patients when compared to CC + CT patients (-1.30 ± 1.32 versus - 0.60 ± 1.05, $p = 0.034$). No association between TRAF6 rs540386 and local bone damage was observed.

Conclusions: This study for the first time ever demonstrated an association between a genetic variant of TRAF6 and low BMD among patients with RA. Further investigations are needed to elucidate the exact role of this variant.

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P04.081.B Association between a functional polymorphism within the IL-17RC gene and idiopathic scoliosis in Bulgarian population

***Svetla Nikolova**[1], Milka Dikova[2], Alexandre Loukanov[3]*

[1]Sofia University, Sofia, Bulgaria, [2]Medical University-Sofia, Sofia, Bulgaria, [3]Saitama University, Saitama, Japan.

Introduction: Several genome wide association studies suggested the common IL-17RC gene variants could take part in the pathogenesis of idiopathic scoliosis. The present case-control study investigated the association between a functional polymorphism, IL-17RC*rs708567 (G/A), and idiopathic scoliosis in a Bulgarian population sample.

Materials and Methods: The association study was performed on 127 patients and 254 controls after obtaining written informed consent. The mean Cobb angle was 53.8° ± 21.2. The mean age of patients was 11.2 ± 2.9 years. The cases were divided into subgroups based on the age of onset, sex, family history, and progression. The genotyping was carried out by TaqMan Real-Time PCR method. The statistical analysis was performed by Pearson's Chi-squared test and Fisher's Exact Test with p-value less than 0.05 as statistically significant.

Results: The frequencies of the variant A allele and AA genotype in the total group of patients and in the subgroup of patients with Cobb angle above 40° were significantly higher than that in the controls ($p < 0.05$). In addition, this case-control study revealed statistically significant association between IL-17RC*rs708567 (G/A) and primary scoliosis in males, females, adolescents, familial and sporadic cases.

Conclusions: The results confirmed previously reported associations between a common variant within IL-17RC and idiopathic scoliosis in Caucasian and Asian population groups and suggested that the molecular marker rs708567 is an independent predisposing and modifying factor of idiopathic scoliosis in different subgroups of Bulgarian patients.This work was supported by MEXT/JSPS KAKENHI T20K05260 and Jikoshunyu Kyoinhaibun-keihi T5452 funded by Saitama University.

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P04.082.C Genotype-phenotype correlation of aberrations at 7q21.2-q21.3 locus in patients affected with isolated or syndromic form of split-hand/foot malformation

***Anna Sowińska-Seidler**[1], Magdalena Socha[1], Anna Materna-Kiryluk[1,2], Aleksander Jamsheer[1,2]*

[1]Department of Medical Genetics, Poznan University of Medical Sciences, Poznań, Poland, [2]Centers for Medical Genetics GENESIS, Poznań, Poland.

Split-hand/foot malformation (SHFM) refers to the group of congenital limb malformations characterized by the absence or hypoplasia of the central rays of the autopods. Eight loci are associated with this clinically and genetically heterogeneous disorder. SHFM type 1 (SHFM1) maps to 7q21.2-q21.3 and occurs in an isolated form, associated with other abnormalities, or as a part of a congenital anomaly syndrome. In most cases SHFM1 results from deletions encompassing the *DLX5/DLX6* genes or their regulatory elements. Herein, we report on five new index cases harboring 7q21.2-q21.3 rearrangements and perform a genotype-phenotype correlation for these individuals and two previously published families of Polish origin affected with SHFM1. Chromosome analysis including conventional GTG banding and array-based comparative genomic hybridization (aCGH) were applied to identify the causative aberrations. Balanced translocations: t(7;12)(q21.2;q21.3) and t(7;10)(q21.2;q22.2) were identified in the most severely affected patient diagnosed with EEC and developmental delay, and in a patient with bilateral ectrodactyly of the hands and feet and hearing loss, respectively. Two other sporadic patients affected with isolated ectrodactyly of the feet carried microdeletions spanning less than 200 kb encompassing the limb-specific enhancers within *DYNC1I1*. Also, a 4.5 Mb deletion of the 7q21.2-

q21.3 region was identified in a sporadic patient diagnosed with EEC syndrome. We present the spectrum of abnormalities in SHFM1 cases that depends on the 7q21.2-q21.3 aberrations breakpoints, deletion size and its gene/regulatory elements content. This work was supported by the grants from the Polish National Science Centre UMO-2016/21/D/NZ5/00064 to A.S-S., UMO-2016/23/N/NZ2/02362 to M.S. and UMO-2016/22/E/NZ5/00270 to A.J.

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P04.083.D SHFM3 caused by a duplication involving *BTRC* but not *POLL* and with possible modifier variants in *FRAS1* and *C2CD3*

Gökhan Nalbant[1], *Aslıhan Tolun*[2]

[1]*Department of Biostatistics and Bioinformatics, Institute of Health Sciences, Acibadem University, Istanbul, Turkey,* [2]*Department of Molecular Biology and Genetics, Istanbul Technical University, Istanbul, Turkey.*

Introduction: Split-hand/foot malformation (SHFM) involves the central rays, the phenotype is very diverse, and six loci are known. SHFM3 (10q24) displays dominant inheritance. Causative duplication includes two genes; BTRC is involved in Wnt signalling cascade by regulating β-catenin levels in limb development and POLL in base excision repair. Whether both genes contribute to the condition has not been elucidated. The disorder varies in severity even among relatives. We investigated a Turkish family afflicted with SHFM3.

Materials and Methods: Exome sequencing for unaffected mother, affected father and two affected daughters was performed to find the causal variant. SNP genotypes were used to detect the disease gene locus and to investigate for any deletion or duplication linked to the malformation.

Results: Linkage analysis revealed a single locus at 10q24.32. All 7 affected individuals investigated carried a maximal 264 kb heterozygous duplication. Very rare *FRAS1*:p.E296K and *C2CD3*:p.A2041V were found in daughters but not father.

Conclusions: We present the smallest duplication causing SHFM3 and involving *BTRC* gene only, indicating that *POLL* does not contribute to the condition. Candidate variants detected could be the genetic factors that aggravate the malformation in siblings and are being investigated for possible relations to pathways regulated by BTRC. Both FRAS1- and C2CD3-deficit phenotypes include autopod malformations. We will also search for modifiers in the second-cousin. Modifier variants could give a clue about why this disorder exhibits variable severity among kin. Supported by the Research Fund of the Istanbul Technical University (2021-42537).

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P04.084.A Is *Xlas* associated with short stature?

Arrate Pereda[1], *Yerai Vado*[1,2], *Karen E. Heath*[3,4,5], *Jesus Pozo-Roman*[6,7,8], *Maria Angeles Santos-Mata*[9], *Elena Artola*[10], *Guiomar Perez de Nanclares*[1]

[1]*Rare Diseases Research Group, Molecular (Epi)Genetics Laboratory, BioAraba Health Research Institute, OSI Araba, Araba University Hospital-Txagorritxu, Vitoria-Gasteiz, Araba, Spain,* [2]*NanoBioCel Research Group, Laboratory of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of the Basque Country UPV/EHU, Vitoria-Gasteiz, Araba, Spain,* [3]*Institute of Medical and Molecular Genetics (INGEMM), La Paz University Hospital, Autonomous University of Madrid, IdiPAZ, Madrid, Spain,* [4]*Skeletal dysplasia Multidisciplinary Unit (UMDE), La Paz University Hospital, Madrid, Spain,* [5]*CIBERER, ISCIII, Madrid, Spain,* [6]*Departments of Pediatrics and Pediatric Endocrinology, Hospital Infantil Niño Jesús University Hospital, Autonomous University of Madrid, Madrid, Spain,* [7]*La Princesa Research Institute, Madrid, Spain,* [8]*CIBEROBN, Institute of Health Carlos III, Madrid, Spain,* [9]*Pediatric Endocrinology Unit, Paediatric Service of the University Hospital de Jerez, Jerez de la Frontera, Cádiz, Spain,* [10]*Paediatric Department, Donostia University, Donostia, Gipuzkoa, Spain.*

Introduction: *GNAS*, located on 20q13.2, is a highly complex imprinted locus from which at least four transcripts (NESP55, GNAS, XL-GNAS and A/B) are generated and, some of them, in an allele specific way. It is well known that heterozygous *GNAS* alterations in either allele are associated with growth impairment, pre- and postnatally. But less is known about the effect of genetic alterations at XLαs *(eXtra Large Gsa)*, encoded by and alternative exon 1 of the paternally expressed long form of *Gsa*.

Patients and Methods: Three independent families in which the probands (P) were referred for short stature (SS) (P1) and pseudopseudohypoparathyroidism (PPHP) or PTH-related protein signalling disorder type 2 (iPPSD2) (P2,P3) were studied by NGS-custom panels. Variant confirmation and cosegregation studies were carried out via Sanger sequencing. Parental origin of the allele was tested by allele-specific RT-PCR amplification, and sequencing.

Results: Three different heterozygous variants of uncertain significance (VUS) were identified in the *XLαs* exon (Table 1). Familial studies suggested cosegregation when the variant was present on the paternal allele.

Conclusion: We describe the first three families in whom XLαs specific exon variants on the paternal allele seem to be associated with short stature and brachydactyly (BD). Additional studies are required. Funding: ESPE RU Grant 2020; ISCIII, Spanish Ministry of Economy and Competitiveness, co-financed by the European Regional Development Fund (PI20/00950); University Basque Country UPV/EHU (PIF17/29).

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P04.085.B Monogenic variants in short stature: use and efficiency of targeted NGS panel in 300 patients

Caroline Michot[1], *Pauline Marzin*[1], *Geneviève Baujat*[1], *Coralie Haudry*[2], *Anne-Laure Tourre*[2], *Julie Steffann*[2], *Sophie Rondeau*[2], *Valérie Cormier-Daire*[1]

[1]*Reference Center for skeletal dysplasia, INSERM UMR1163, Paris Descartes-Sorbonne Paris Cité University, Imagine Institute, Necker-Enfants Malades Hospital, Paris, France,* [2]*Molecular Genetic Unit, Paris Descartes-Sorbonne Paris Cité University, Necker-Enfants Malades Hospital, Paris, France.*

Short stature is a frequent reason for paediatric consultations. After clinical, endocrinological and radiological explorations, even patients shorter than -2,5 SD remain without diagnosis. Next generation sequencing (NGS) has improved the ability to offer testings for heterogeneous conditions. We report the analysis of 300 patients with short stature.

A targeted NGS panel was designed to sequence 150 skeletal dysplasia genes. Amplicons were captured by a custom SureSelect kit (Agilent) and sequenced on HiSeq (Illumina). Annotation and analysis of variants were realized by a homemade interface

(PolyWeb). Results were discussed with the physicians of the reference center for Skeletal Dysplasias.

Pathogenic variants were identified in 48 patients (16%) and variants of unknown significance (VOUS) in 18 cases (6%), involving 8 different genes. Pathogenic variants were identified in: *NPR2* (n = 16), *ACAN* (n = 11), *FGFR3* (n = 8), *IHH* (n = 8) and *SHOX* (n = 5). Patients had neither body disproportion, nor Madelung deformation. X-rays showed no or minor abnormalities, such as bone age anomalies, slightly curved radius or stocky long bones. VOUS variants involved the same genes, and also *COL9A2* (n = 1), *COL11A1* (n = 2) and *COL11A2* (n = 2). These last variants were classified as class 3, as patients displayed no epiphyseal dysplasia.

Targeted NGS allowed molecular elucidation in 16 % of patients with proportionate short stature and minor skeletal features. These results highlight the impact of monogenic variants in short stature patients, involving a few number of genes, and the efficiency of targeted NGS in this indication. Molecular elucidation is important for the assessment of therapeutic options for these patients.

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P04.086.C Identification and tissue-specific characterization of novel SHOX-regulated genes highlights *SOX* family members among other genes

Sandra Hoffmann[1], *Ralph Roeth*[1], *Sabrina Diebold*[2], *Jasmin Gogel*[1], *Steffen Just*[2], *Gudrun A. Rappold*[1]

[1]*Institute of Human Genetics, Heidelberg, Germany,* [2]*Clinic for Internal Medicine II, Molecular Cardiology, Ulm, Germany.*

SHOX-deficiency causes a spectrum of clinical phenotypes related to skeletal dysplasia and short stature including Léri Weill dyschondrosteosis, Langer mesomelic dysplasia, Turner syndrome, as well as idiopathic short and tall stature. SHOX controls chondrocyte proliferation and differentiation, bone maturation, as well as cellular growth arrest and apoptosis via transcriptional regulation of its direct target genes *NPPB*, *FGFR3*, and *CTGF*. However, our understanding of SHOX-related pathways is still incomplete. To elucidate the underlying molecular mechanisms and to better understand the broad phenotypic spectrum of *SHOX*-deficiency, we analyzed differentially expressed genes in human fibroblasts (NHDF), where *SHOX* is expressed at detectable level. Twenty-three putative target genes were selected for further validation by whole-body and tissue-specific characterization (head, heart and pectoral fins) in developing *shox*-deficient zebrafish embryos. Physiological relevance was confirmed for the majority of these genes in pectoral fins and network-based analysis showed that 20/23 genes act in a common network. Interestingly, several *sox* family members (*sox5*, *6*, *8* and *18*) were shown to be significantly deregulated in *shox*-deficient pectoral fins among other genes including *nppa*, *nppc*; *cdkn1a*, *cdkn1ca*, *cyp26b1*, *cyp26c1*, highlighting the important role of gene family members in *shox*-related growth disorders. Our results provide novel insights into the genetic pathways and molecular events leading to the clinical manifestation of *SHOX*-deficiency.

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P04.087.D Exome analysis of prenatal and postnatal cases referred with skeletal dysplasia

Andrea Haworth[1], *Tessa Homfray*[2], *Suzanne Drury*[1], *Rand Dubis*[1], *Meriel McEntagart*[2], *Katrina Tatton-Brown*[2], *Helen Savage*[1], *John Filby*[1], *Nicholas J. Lench*[1], *Louisa Ive*[1], *Eva Serra*[1], *Natalie Trump*[1], *Nayana Lahiri*[2], *Janna Kenny*[2], *Frances Elmslie*[2], *Phil Ostrowski*[2], *Esther Dempsey*[2], *John Short*[2], *Charlene Crosby*[2], *Christine M. Hall*[2], *Sahar Mansour*[2]

[1]*Congenica Limited, Cambridge, United Kingdom,* [2]*St George's Hospital NHS Trust, London, United Kingdom.*

Skeletal dysplasias (SD) are rare disorders representing approximately 5% of all congenital anomalies. They are highly heterogeneous and clinical findings are often non-specific, so accurate diagnosis often relies on expert interpretation of radiological findings. Until recently treatments have been limited but development of new therapeutics has highlighted the need to provide accurate and timely diagnosis. We describe the genomic and phenotypic findings of SD cases referred to clinical genetics over an approximately 2.5-year period. All cases underwent exome sequencing (ES) as part of a service provided by Congenica and the South West Thames Regional Genetics Service, London. 53 cases were referred with a clinical diagnosis of possible SD and a molecular diagnosis was obtained in 49% (26/53). A higher diagnostic yield was observed in prenatal (64%, 16/25) rather than postnatal cases (35.7%, 10/28). Causal variants were identified in 26 genes. Variant types identified included those impacting both coding and non-coding regions (5'UTR) and CNVs. The molecular diagnosis was not always concordant with the suspected clinical diagnosis, particularly in a prenatal setting and the advantages of undertaking analysis of all skeletal dysplasia genes in these cases is illustrated in two cases (*SOX9* and *GNPTAB*). Accurate molecular diagnosis has directly influenced patient management, including 2 cases with spondylocarpotarsal synostosis where surveillance was initiated and 1 case of prenatal hypophosphatasia where diagnosis provided appropriate management of pregnancy and access to therapeutic intervention. Furthermore, in one family the identification of the underlying molecular cause has expanded the phenotypic spectrum of disease (cartilage-hair hypoplasia).

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Proband	Features	Other affected family members	Parental origin	Custom panel	XLαs NM_080425.3	NP_536350.2	ACMG
1	SS	father, paternal grandfather	Paternal	Skeletal dysplasia (385 genes)	c.1043G>A	p.(Arg348Gln)	PM2; BP4 (7 vs 2) VUS
2	SS, BD	sister, father (only SS)	Paternal	iPPSD and related disorders (92 genes)	c.1343A>C	p.(Asp448Ala)	BP4 (13 vs 0) VUS?
3		none	*De novo*? (ongoing)		c.79G>A	p.(Glu27Lys)	PM2, BP4 (9 vs 4) VUS

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P04.088.A What are key parameters for obtaining the most likely clinical diagnosis from the wide phenotypic spectrum of skeletal dysplasia in patients with previously identified disease-causing gene variant

Marija Mijovic[1], *Bojan Bukva*[2,3], *Jelena Ruml Stojanovic*[1], *Aleksandra Miletic*[1], *Brankica Bosankic*[1], *Hristina Petrovic*[1], *Goran Cuturilo*[1,3]

[1]*University Children's Hospital, Department of Clinical Genetics, Belgrade, Serbia,* [2]*University Children's Hospital, Department of Orthopedic Surgery, Belgrade, Serbia,* [3]*Faculty of Medicine, University of Belgrade, Belgrade, Serbia.*

Introduction: It is known that alteration of several genes associated with skeletal disorders could lead to multiple, highly variable phenotypes. Aim of present study was to establish the crucial clinical and/or genetic parameters for obtaining the diagnosis in three patients with previously unclassified skeletal dysplasia in whom the causal gene variant was identified.

Materials and Methods: Genetic testing was performed using whole exome sequencing (WES). Detailed analysis of genotype-phenotype correlation was performed by the team of geneticist and child orthopedist. Contribution of following parameters was assessed: specific radiological and orthopedic signs; existence of associated disorders; adult phenotype in familial cases; phenotype of the patient with the same variant from the literature if available, and gene localization of the identified variant.

Results: Case 1: Infant with severe nonfamilial skeletal dysplasia, with congenital arthrogryposis and multiple associated problems. WES result: *TRPV4*: heterozygous pathogenic missense variant c.806G>A, p.Arg269His. Clinical diagnosis: Metatropic dysplasia. Case 2: Child with familial skeletal dysplasia with short stature and coxa vara. WES result: *COL2A1*: heterozygous pathogenic missense variant c.1681G>A, p.Gly561Ser. Clinical diagnosis: Spondyloepiphyseal dysplasia with metatarsal shortening (Czech dysplasia). Case 3: Young child with familial skeletal dysplasia with short stature, joint laxity and other skeletal involvement. WES result: *COMP*: heterozygous likely pathogenic missense variant c.1293C>A, p.Asp431Glu. Clinical diagnosis: Multiple epiphyseal dysplasia (Fairbank type).

Conclusion: Multidisciplinary approach could facilitate the establishment of the most likely clinical diagnosis in patients with skeletal disorders with previously identified causative gene variant. Key parameters for obtaining the clinical diagnosis differ from gene to gene.

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P04.089.B A previously unreported pathogenic variant in *TAB2* in a patient with polyvalvular heart dystrophy, short stature and dysmorphism

Nis Brix[1,2], *Rikke Christensen*[1], *Susanne W. Grotkjær*[3], *Dorte G. Nielsen*[4], *Kasper J. Kyng*[5], *Pernille A. Gregersen*[1,6]

[1]*Department of Clinical Genetics, Aarhus University Hospital, Aarhus N, Denmark,* [2]*Department of Public Health, Aarhus University, Aarhus, Denmark,* [3]*Department of Pediatrics, Herning Hospital, Herning, Denmark,* [4]*Department of Cardiology, Aarhus University Hospital, Aarhus N, Denmark,* [5]*Department of Pediatrics and adolescence, Aarhus University Hospital, Aarhus N, Denmark,* [6]*Centre for Rare Diseases, Department of Pediatrics and Adolescence, Aarhus University Hospital, Aarhus, Denmark.*

Introduction: TGFβ-activated kinase 1-binding protein 2 *(TAB2)* encodes a scaffold protein that forms the TAK1-TAB complex involved in proliferation, differentiation and myocardial homeostasis. Pathogenic variants are associated with autosomal dominant congenital heart defects (OMIM #614980). We describe a 5-year-old girl and her mother with a previously unreported frameshift variant in *TAB2* identified by whole exome sequencing (WES). Clinical report: The girl was born by elective caesarean section (gestational age 39+0, birth weight 2594g). She was followed for growth retardation, and at 4.5 years, she was -3SD for height and weight. She had mild insufficient dysplastic atrioventricular valves and joint hypermobility. She was dysmorphic with fifth finger clinodactyly, pes planus, mild hypertelorism, epicanthal folds, mild midface hypoplasia, frontal bossing, bulbous nasal tip and broad mouth. The mother has short stature (-2.5SD), a dysplastic mitral valve with mild insufficiency, and has previously been treated for supraventricular tachycardia. Several other family members on the mother's side have short stature and variable cardiac abnormalities.

Methods and Results: Using WES performed on DNA from the patient and both parents, we analysed data as a trio with focus on maternally inherited variants and found heterozygosity for a previously unreported maternally inherited class 4 variant (ACMG guidelines) in*TAB2* (NM_015093.5:c.[604_605dup];[=], p.(Pro203Hisfs*41)). Segregation analysis in the family is ongoing.

Discussion: Our findings, especially the precise clinical description of an adult person (the mother) and the extensive pedigree, add to the phenotypic spectrum of a *TAB2*-related systemic connective tissue disorder with polyvalvular dystrophy, short stature and dysmorphism.

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P04.090.C Evidence that ciliary genes contribute to non-syndromic familial tall stature

Birgit Weiss[1], *Birgit Eberle*[1], *Ralph Röth*[1], *Christiaan de Bruin*[2], *Julian C. Lui*[3], *Katrin Hinderhofer*[1], *Jeffrey Baron*[3], *Jan M. Wit*[2], ***Gudrun A. Rappold***[1]

[1]*Institute of Human Genetics, Heidelberg, Germany,* [2]*Leiden University Medical Center, Leiden, Netherlands,* [3]*National Institute of health, Bethesda, MD, USA.*

Human growth is a complex trait. A considerable number of gene defects have been shown to cause short stature, but there are only few examples of genetic causes of non-syndromic tall stature. Besides rare variants with large effects and common risk alleles with small effect size, oligogenic effects may contribute to this phenotype. Exome sequencing was carried out in a tall male (height 3.5 SDS) and his parents. Filtered damaging variants with high CADD scores were validated by Sanger sequencing in the trio and three other affected and one unaffected family members. Network analysis was carried out to assess links between the candidates, and the transcriptome of murine growth plate was analyzed by microarray as well as RNA Seq. Heterozygous gene variants in *CEP104, CROCC, NEK1, TOM1L2* and *TSTD2* predicted as damaging were found to be shared between the four tall family members. Three of the five genes *(CEP104, CROCC* and *NEK1)* belong to the ciliary gene family. All genes are expressed in mouse growth plate. Pathway and network analysis indicated close

functional connections. Together, these data expand the spectrum of genes with a role in linear growth and tall stature phenotypes.

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P04.091.D Compound heterozygous mutations in ERCC2 are associated with trichothiodystrophy type 1

Fahrettin Duymus, *Deniz Esin, Busra Goksel Tulgar, Nadir Kocak, Tulin Cora*

Selcuk University, Konya, Turkey.

A 9-month-old male patient was admitted to our clinic with sparse hair, developmental delay and recurrent infections. He was the first child of child of non-consanguineous Turkish parents. In the patient's anamnesis, it was learned that there was a rash on her skin at birth.İchthyosis and mild scaling were reported on the scalp, trunk, and lower extremities of the patient from 1 month of age. In the evaluation of the patient, there were short brittle hair, dry skin, dystrophic tooth structure, and hyperkeratosis in the feet. Whole exome sequencing analysis was performed from the patient who had ichthyosis and frequent infections.ERCC2: c.2164C>T and ERCC2:c.1867dup heterozygous mutations were detected. The clinical findings of the patient were also compatible with trichothiodystrophy (TTD). TTD is a rare genetic disease characterized by brittle hair, ichthyosis, and recurrent infections. Molecular genetic evaluation is very useful in the diagnosis of TTD. ERCC2: c.2164C> T mutation was previously reported as compound heterozygous with pathogenic p. (Arg616Pro) variant in a patient diagnosed with TTD in 1994 by Broughton et al. To the best of our knowledge the ERCC2: c.1867dup variant has not been previously reported in the literature and is a frame shift-type variant. it has never been described before that the compound heterozygosity of these mutations causes photosensitive TTD. He was diagnosed to have photosensitive TTD type 1 and his mother and father are the carriers. Genetic counseling was given to the family of the patient. A schedule was prepared for regular clinic follow-up of the patient.

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P04.093.B An exceptional biallelic N-terminal frameshift mutation in *ZMPSTE24* leads to non-lethal progeria due to utilization of a downstream alternative start codon

Lukas Kaufmann[1], *Jasmin Blatterer*[1], *Erich Schaflinger*[1], *Aiman S. Khan*[2], *Lisa Auinger*[1], *Benjamin Tatrai*[1], *Sumra W. Abbasi*[3], *Muhammad Z. Ali*[2], *Ansar A. Abbasi*[4], *Ali Al Kaissi*[5], *Klaus Wagner*[1], *Muzammil A. Khan*[2], *Christian Windpassinger*[1]

[1]*Diagnostic & Research Institute of Human Genetics, Graz, Austria,* [2]*Gomal Centre of Biochemistry and Biotechnology, D. I. Khan, Pakistan,* [3]*NUMS Department of Biological Sciences, Rawalpindi, Pakistan,* [4]*Department of Zoology, Mirpur, Pakistan,* [5]*Ludwig Boltzmann Institute of Osteology, Vienna, Austria.*

Introduction: Mandibuloacral dysplasia with type B lipodystrophy (MADB) is a rare autosomal recessive disorder of premature aging, associated with severe abnormalities in bone, skin and adipose tissue. The main underlying genetic cause of MADB are homozygous or compound heterozygous missense mutations in the *ZMPSTE24*-gene. Biallelic loss-of-function mutations in *ZMPSTE24*, however, have been associated with lethal restrictive dermopathy (RD), which leads to death within the first weeks of life. In the present study, a large consanguineous Pakistani family segregating MADB was recruited for molecular investigation in order to identify the underlying genetic cause.

Materials and Methods: The disease was diagnosed through clinical features of MADB, assisted by radiologic and biochemical tests. For genetic analysis whole exome sequencing (WES) was performed, followed by homozygosity mapping and variant annotation using VarSeq™ v2.2 (Golden Helix, Inc.).

Results: Whole exome sequencing revealed a novel N-terminal homozygous frameshift mutation NM_005857:c.28_29insA, p.(Leu10Tyrfs*37) in *ZMPSTE24* segregating with the disease phenotype. Remarkably, the potential loss-of-function mutation results in a non-lethal phenotype in our patients. A more in-depth analysis of the mutation revealed that the observed one base pair insertion creates a novel downstream in-frame start codon.

Conclusions: To our knowledge, this is the first report of a biallelic loss-of-function mutation not implicated in lethal RD. We propose a rescue mechanism involving the usage of a gained in-frame downstream start codon, which could potentially avoid frameshift and lead to residual enzymatic function. These findings might be crucial for the interpretation of far N-terminal mutations in complex genotype-phenotype correlations.

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P05 Cardiovascular Disorders

P05.001.C Segregation of rs897543876 in *BMP4* gene in family with nonsyndromic aortic dilatation

Zuzana Capkova[1,2], *Jana Petrkova*[1,2], *Pavlina Capkova*[1,2], *Lucie Punova*[1,2], *Martin Prochazka*[1,2], *Josef Srovnal*[1,3]

[1]*Department of Medical Genetics, University Hospital Olomouc, Olomouc, Czech Republic,* [2]*Department of Medical Genetics, Faculty of Medicine and Dentistry, Palacký University Olomouc, Olomouc, Czech Republic,* [3]*Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University Olomouc, Olomouc, Czech Republic.*

Background: Aortic dilatation is the most common pathological involvement of the thoracic aorta. This aberration can arise by various causes such as congenital connective tissue disorder (CCTD), injury or inflammation and is accompanied by bicuspid aortic valve, arterial hypertension and atherosclerosis. CCTD were associated with increase of aortic dilatation but genetics of nonsyndromic aortic dilatation is still unknown. Case report: Here we report family with aortic dilatation. Proband was genetically tested for CCTD in his 61 years. Aortic dilatation was observed and his echocardiography showed bicuspid aortic valve. Hypertension was treated in this patient. His brother had aortic dilatation and aortic insufficiency. Daughter of proband was observed for anxiety. Daughter and son of proband were without aortic dilatation.

Method and result: Proband was consulted by geneticist and biological materials was collected with his informed consent to genetic testing. Marfan syndrome was excluded by sequencing of *FBN1*. Also DiGeorge syndrome was excluded by multiplex ligation-dependent probe amplification (MLPA). Moreover MLPA revealed heterozygously deleted one probe in *BMP4*. Sequencing of probe area showed unknown variant rs897543876 (NM_001202.6:c.-144C>T). Brother and daughter of proband also carried rs897543876.

Discussion and conclusion: rs897543876 is unknown variant which appears in 0.012 %-0.014 % worldwide and predicted as

variant with moderate pathogenicity according to prediction tools. The variant is located in 5´UTR and may downregulated expression of *BMP4*. However, future studies are necessary for confirmation of rs897543876 pathogenic role. Acknowledgment: MH CZ—DRO FNOL 00098892, IGA UP LF_2020_007, IGA UP LF_2020_018, TACR TN01000013, No. CZ.02.1.01/0.0/0.0/16_019/0000868, LM2018132, A-C-G-T, CZ.02.1.01/0.0/0.0/16_026/0008448.

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P05.002.D Malignant ventricular arrhythmia in a Czech representative cohort of sudden cardiac death (SCD) victims and cardiac arrest survivors (CAS) aged 1 - 65 years: results of a candidate gene panel-based sequencing strategy

Pavel Votypka[1], *Petra Peldova*[1], *Stepanka Pohlova-Kucerova*[2], *Marketa Kulvajtova*[3], *Petr Peichl*[4], *Marek Sramko*[4], *Andrea Gregorova*[5], *Terezia Tavacova*[6], *Jana Petrkova*[7], *Martin Dobias*[8], *Petr Tomasek*[9], *Vlastimil Vancura*[10], *Milan Macek Sr.*[1], *Milan Macek Jr.*[1], *Jan Janousek*[11], *Josef Kautzner*[4], *Alice Krebsova*[4]

[1]*Department of Biology and Medical Genetics, 2nd Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic,* [2]*Department of Forensic Medicine, University Hospital Hradec Kralove, Hradec Kralove, Czech Republic,* [3]*Institute for Forensic Medicine, 3rd Faculty of Medicine, Charles University, Prague, Czech Republic,* [4]*Institute for Clinical and Experimental Medicine, Prague, Czech Republic,* [5]*Department of Biology and Medical Genetics, Faculty Hospital Ostrava, Ostrava, Czech Republic,* [6]*Children´s Heart Center, Faculty Hospital Motol, Prague, Czech Republic,* [7]*1st Department of Internal Medicine - Cardiology and Laboratory of Cardiogenomics, Faculty Hospital Olomouc and Palacky University, Olomouc, Czech Republic,* [8]*11Institute of Forensic Science and Medical Law, Faculty Hospital Olomouc and Palacký University, Olomouc, Czech Republic,* [9]*Department of Forensic Medicine, University Hospital Bulovka., 2nd Faculty of Medicine, Charles University, Prague, Czech Republic,* [10]*Department of Cardiology, Faculty Hospital Pilsen, Pilsen, Czech Republic,* [11]*Children´s Heart Centre, 2nd Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic.*

Introduction: Hereditary cardiomyopathy and arrhythmic syndromes are associated with an increased risk of malignant ventricular arrhythmia leading to SCD or cardiac arrest. Genetic testing confirms clinical / autopsy diagnosis and enables stratified care.

Patients and Methods: Altogether 166 CAS (71 female and 97 males) and 76 SCD victims (25 female and 51males) were analysed. In the CAS group 103 cases have dg. of idiopathic ventricular arrhythmia (iVF), 34 arrhythmogenic cardiomyopathy (ACM; of which 27 right -/ 7 left predominant) and 2 have hypertrophic cardiomyopathy (HCM). The SCD group comprises 11 cases with post mortem dg. of hypertrophic - (HCM) /dilated- (DCM), 16 arrhythmic cardiomyopathy (ACM), 20 sudden arrhythmic death (SADS) and 18 sudden unexplained death cases (SUD). Massively parallel sequencing (MiSeq platform; Illumina.com) utilised a custom-made panel with 100 candidate genes (SOPHiA Genetics; Switzerland). Presence of Class 4-5 variants was validated by Sanger sequencing and via family segregation analyses.

Results: The cumulative detection rate of Class 4-5 variants in CAS was in 49/166 (30 %) and in SCD victims 18/76 (22 %). The most commonly affected gene was *PKP2* (12/67 variant-positive cases), followed by *KCNH2* (8/67), *RYR2* (7/67), *TTN* (7/67) and *SCN5A* (6/67).

Conclusion: In a representative Czech cohort, cardiac arrest could be frequently explained by a molecular cause. Malignant arrhythmias in CAS and SCD victims are mainly due to pathogenic variants in genes associated with ACM, LQT2, CPVT and LQT3. Supported by Ministry of Health of the Czech Republic, grant Nr. NV18-02-00237.

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P05.004.B Analysis of the effect of periodontopathogens on the molecular mechanisms of atherosclerosis. Systematic review

Talia Yolanda Marroquin[1], *Sandra Guauque-Olarte*[2]

[1]*Universidad Cooperativa de Colombia, Pasto, Colombia,* [2]*Universidad Cooperativa de Colombia, Medellin, Colombia.*

Introduction: The role periodontal pathogens in atherosclerosis has not been fully understood. The aim of this systematic review was to describe the effect of periodontopathogens on the molecular mechanisms involved in atherosclerosis.

Materials and methods: This systematic review followed the Cochrane and PRISMA guidelines. Screened databases were PubMed, Science Direct, and Lilacs. Original studies published from January 2015 to August 2020 about periodontitis and atherosclerosis were included. A tool for quality assessment, based on STROBE checklist, was applied.

Results: A total of 722 records were collected, 33 papers were selected for full-text reading. *Porphyromonas gingivalis,Tannerella forsythia, Fusobacterium nucleatum*, and *Treponema denticola*, were able to disseminate from the oral cavity and invade atherosclerotic plaque in humans. In mice sera, these bacteria significantly increased the expression of 25 genes or proteins belonging to apoptosis, TLR signalling, TGF-beta, inflammation, and angiogenesis pathways. Ten papers reported that *P. gingivalis* and *Eikenella corrodens* significantly increased the expression of 42 biomarkers associated with atherosclerosis development and progression pathways such as apoptosis, cytochrome-c, inflammation, entdothelin, B cell activation, vascular endothelial growth factor, cell adhesion, and toll like receptor pathways, in HAECs, HUVECs, and AoSMCs. *Filifactor alocis* significantly increased angiogenesis-related biomarkers. *P. gingivalis* significantly decreased the expression biomarkers associated with antiapoptotic mechanisms in HCAECs, HASMSs, HUVECs.

Conclusions: Data-analysis revealed the significant effect of some periodontopathogens on proatherogenic mechanisms. Network analysis will be performed on this data. It is important to study the effect of other periodonthopathogens in atherogenesis. SGO received funding by CONADI and TYM by Minciencias.

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P05.005.C Somatic mosaicism of human coronary artery cells in atherosclerosis

Aleksei Zarubin, *Anton Markov, Maria Nazarenko*

Research Institute of Medical Genetics, Tomsk National Research Medical Center, Tomsk, Russian Federation.

Introduction: Accumulation of somatic mutations is usually associated with aging, and age-associated diseases, e.g., atherosclerosis. We assessed the scale and cell specificity of this

phenomenon in the human coronary arteries affected by atherosclerosis using single cell RNA sequencing (scRNA-seq) data.

Materials and Methods: We used public dataset (PRJNA544957). Briefly, coronary arteries from four explanted heart affected by atherosclerosis were dissociated and used for scRNA-seq. Sample datasets were pooled for clustering and cell type determination. Single cells were genotyped using cellSNP-lite. Total cell genotype served as reference for every sample separately. We counted the number of cells with somatic mutations in each cluster.

Results: The maximal proportion of mosaic cells was specific for plasma cells (53.9%). Somatic variants were found to a greater extent in genes of HLA receptor family and cytochrome b-245. Smooth muscle cells were the second cluster in terms of somatic mosaicism frequency (13.5%). They also contained alterations in HLA genes, but also in the genes of metallothionein and apolipoprotein D. Somatic heterogeneity of macrophages was found in actin and tubulin genes, in addition to genes of previously described cell types. At the same time, the proportion of cells containing two or more somatic variants among smooth muscle cells was significantly less (0.3%) than in plasma cells (30%).

Conclusion: We have discovered somatic mosaicism in coronary artery cells affected by atherosclerosis. Different cell types have different levels of mosaicism. At the same time, there are genetic variants both shared by all cells and specific for individual cell types.

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P05.006.D A Polygenic Risk Score for Atrial Fibrillation in a Hispanic/Latino Population

Brandon Chalazan[1], *Christina Lee*[2], *Valerie Morrill*[2], *Sara Darbar*[3], *Aylin Ornelas-Loredo*[3], *Arvind Sridhar*[4], *Martha Daviglus*[3], *Patrick Ellinor*[2], *Dawood Darbar*[3]

[1]*University of British Columbia, Vancouver, BC, Canada,* [2]*Broad Institute, Boston, MA, USA,* [3]*University of Illinois, Chicago, IL, USA,* [4]*University of Illinois at Chicago, Chicago, IL, USA.*

Background: Previous genetic studies for atrial fibrillation (AF) have identified common and rare variants associated with AF. Recently, polygenic risk scores (PRSs) have been developed for AF almost exclusively in patients of European ancestry. Here, we performed a candidate single nucleotide polymorphism (SNP) on a large cohort of Hispanic/Latino individuals determine the combined impact of genetic variants.

Purpose: To generate a PRS to estimate the risk of AF in a Hispanic/Latino cohort.

Methods: We prospectively enrolled 713 participants from the UIC AF Registry and UIC Cohort of Patients, Family and Friends. The cohort was genotyped for the top 9 known AF risk alleles and a PRS was developed using these SNPs. The cohort was stratified into quartiles of low: 0-20%; intermediate: 20-80%; and high: 80-100% genetic risk.

Results: The study cohort consisted of 625 Hispanic/Latino subjects with a mean age 46.0 ± 13.5 years and 57% were male. Patients in the intermediate and high genetic risk group were associated with a 2.50-fold (odds ratio [OR] 2.50; 95% confidence interval [CI]: 0.92-6.81; $P = 0.07$) and 2.70-fold (OR 2.70; 95% [CI]: 1.13-6.43; $P = 0.02$) increase in AF risk compared to the low risk group.

Conclusions: We show that a PRS is capable of predicting and risk stratifying AF patients in Hispanic/Latinos populations. Further studies will explore the impact of additional AF loci on risk stratification and determine if PRSs can be integrated into clinical practice.

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P05.007.A Mendelian randomization suggests a causal effect of abdominal obesity on postprandial lipemia

Malene Revsbech Christiansen[1], *Torben Hansen*[1], *Thorkild I. A. Sørensen*[1,2], *Niels Grarup*[1], *Mario García Ureña*[1], *Dmitrii Borisevich*[1], *Jean-Michel Oppert*[3], *José Alfredo Martínez Hernandéz*[4,5], *Ellen Blaak*[6], *Tuomas Oskari Kilpeläinen*[1]

[1]*Novo Nordisk Foundation Center for Basic Metabolic Research, Copenhagen N, Denmark,* [2]*Department of Public Health, Section of Epidemiology, University of Copenhagen, Copenhagen, Denmark,* [3]*Nutrition Department, Pitie-Salpêtrière Hospital, Assistance Publique Hôpitaux de Paris, Sorbonne University, Paris, France,* [4]*Department of Nutrition, Food Sciences, and Physiology, Center for Nutrition Research, University of Navarra, Pamplona, Spain,* [5]*Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y Nutrición, Instituto of Health Carlos III, Madrid, Spain,* [6]*Department of Human Biology, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University, Maastricht, Netherlands.*

Introduction: High postprandial lipemia is associated with an increased risk of cardiovascular disease, independent of fasting lipid levels. Abdominal and gluteofemoral fat handle lipoproteins differently, which could affect postprandial lipemia and thus cardiovascular risk. We aimed to study the causal influences of body fat distribution on postprandial lipemia after a high fat meal, using Mendelian randomization.

Materials and methods: A total of 764 adults with obesity from seven European countries consumed a liquid high fat meal. Postprandial concentrations of triglycerides, glycerol, free fatty acids, and the cholesterol component of remnant-like particles (RLP), LDL and HDL were measured for 3 hours. Waist-hip ratio adjusted for BMI (WHR_{adjBMI}) was instrumented using a genetic score of 442 independent WHR_{adjBMI}-associated genetic variants. Two-stage least squares regression analyses were used to assess causal associations of WHR_{adjBMI} with postprandial lipid levels. Linear regression analyses were used to assess associations of waist circumference and hip circumference adjusted for BMI (WC_{adjBMI} and HC_{adjBMI}) with postprandial lipid levels.

Results: Instrumental variable analyses suggested that WHR_{adjBMI} is causally associated with higher postprandial lipemia after a high fat meal, including higher postprandial levels of triglyceride ($P = 0.044$) and RLP cholesterol ($P = 0.020$). WC_{adjBMI} and HC_{adjBMI} showed directionally opposite effects: WC_{adjBMI} was associated with higher levels of triglyceride and RLP cholesterol and HC_{adjBMI} with lower levels (P for difference $= 1.67 \times 10^{-5}$ and 5.64×10^{-4}, respectively).

Conclusion: Our results suggest that higher abdominal fat deposition is causally associated with higher postprandial lipemia. Furthermore, gluteofemoral fat deposition may show the opposite effect.

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P05.008.B A FAIR Brugada Syndrome data repository to facilitate cardiogenetic research

Marta M. Tébar Ruiz[1,2], *Dorien Daneels*[1,3], *Gudrun Pappaert*[4], *Elyssa Cannaerts*[1], *Thomy de Ravel*[1], *Carlo de Asmundis*[4], *Juan Sieira*[4], *Ann Nowé*[2,5], *Pedro Brugrada*[4], *Catharina Olsen*[1,2,3], *Tom Lenaerts*[2,5,6], *Sonia Van Dooren*[1,2,3]

[1]*Centre for Medical Genetics, research group Reproduction and Genetics, research cluster Reproduction, Genetics and Regenerative Medicine, Vrije Universiteit Brussel (VUB) and Universitair Ziekenhuis Brussel (UZ Brussel), Brussels, Belgium,* [2]*Interuniversity Institute of Bioinformatics in Brussels (IB)2, Brussels, Belgium,* [3]*Brussels Interuniversity Genomics High Throughput core (BRIGHTcore), VUB-ULB, Brussels, Belgium,* [4]*Heart Rhythm Management Centre, Vrije Universiteit Brussel (VUB) and Universitair Ziekenhuis Brussel (UZ Brussel), Brussels, Belgium,* [5]*Vrije Universiteit Brussel, Artificial Intelligence Lab Brussels, Brussels, Belgium,* [6]*Machine Learning Group, Université Libre de Bruxelles (ULB), Brussels, Belgium.*

Introduction: Brugada Syndrome (BS) is a rare cardiac inheritable disorder associated with a high-risk of sudden cardiac death, which may be the first symptom, making essential its detection in pre-symptomatic individuals. Drug-induced ECG monitoring and genetic testing provide powerful early-warning systems. However, in approximately 70% of the cases no causative variant(s) can be identified. In addition, comparisons across multiple centres are currently difficult as so far, no registry with clinical information and causative genetic variants has been made available to the research community. In this study we provide a first structured repository for BS, following FAIR data principles and data protection regulations, in order to facilitate comparisons and further clinical, genetic and AI-driven research.

Methods and results: *Universitair Ziekenhuis Brussel* (UZB) has collected data for BS families since 1992. We manually curated clinical data of patients who consented further research (157 individuals). In addition, we (re-)classified variants for mutations found in the BS and primary cardiac arrhythmia associated genes using Hofman (PMID: 23963746) and ACMG classification guidelines (PMID: 25741868), to highlight differences in classification algorithms and validate the used methodology.

Conclusions: Combining clinical and genetic data is essential to find phenotype-genotype correlations, especially in cases like BS where different causative variants may lead to different severity grades. This new UZB BS repository is intended to boost the integration of data analysis coming from different groups, ensuring a better understanding and diagnosis of BS patients.This initiative is supported by IMAGica IRP project grant of the Vrije Universiteit Brussel.

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P05.009.C Mutations in structural genes in Brugada Syndrome

Marianna Farnè[1], *Rita Selvatici*[1], *Cristina Balla*[2], *Mauro Biffi*[3], *Elia De Maria*[4], *Cecilia Trabanelli*[1], *Alice Margutti*[1], *Matteo Bertini*[2], *Alessandra Ferlini*[1], *Francesca Gualandi*[1]

[1]*Unit of Medical Genetics, Deparment of Medical Sciences, University of Ferrara, Ferrara, Italy,* [2]*Cardiology Department, University Hospital Sant'Anna of Ferrara, Ferrara, Italy,* [3]*Department of Cardiology, University Hospital of Bologna Sant'Orsola-Malpighi, Bologna, Italy,* [4]*Cardiology Unit, Carpi Ramazzini Hospital, Carpi (Modena), Italy.*

Introduction: Brugada syndrome (BrS) is a hereditary disease with high allelic/genetic heterogeneity and associated with the risk of ventricular arrhythmias and sudden cardiac death. This clinical entity is classically described as an arrhythmic condition occurring in a structurally normal heart, but this assumption has been recently contradicted by several observations, which suggest a link between BrS and structural cardiomyopathies.

Materials and methods: We studied a cohort of patients with spontaneous or drug-induced type 1 EKG pattern, variably associated with symptoms and a positive family history by a Next Generation Sequencing panel approach, including both channelopathies and cardiomyopathies genes.

Results: We identified in eleven subjects (13.8%) likely pathogenic/pathogenic variants in genes associated with arrhythmogenic (AC) or hypertrophic (HCM) cardiomyopathy. Four mutations were identified in the two major HCM genes: missense changes p. Arg783His and p.Val1213Met in *MYH7*, a frameshifting p.Lys1065Glnfs*12 and a splicing c.1458-1G>A variations in *MYBPC3*. All of these mutations are known being associated to HCM. Nevertheless, only the patient carrying the *MYBPC3* splicing mutation showed clinical evidence of structural cardiomyopathy.

Conclusions: Our study showed genotypic overlap between BrS and structural cardiomyopathies, including HCM. This observation supports Brugada type 1 ECG pattern as a possible early sign of an occult structural heart disease, with implications in clinical management and genetic counselling.

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P05.010.D Congenital heart defects in Noonan syndrome and PTPN11 muta□onCongenital heart defects in Noonan syndrome and PTPN11 mutation

Laura Claudia Popa[1], *Nicoleta Andreescu*[1,2], *Simona Farcas*[2], *Adela Chirita-Emandi*[1,2], *Maria Puiu*[1,2]

[1]*Louis Turcanu Children Hospital, Timisoara, Romania,* [2]*Victor Babes University, Timisoara, Romania.*

Background: Noonan syndrome is a common autosomal dominant RASopathy disorder clinically characterized by facial dysmorphism, congenital heart disease, short stature and molecularly characterized by mutations in most common genes: PTPN11, SOS1, RAF1, RIT1, KRAS. Mutations in the PTPN11 are the most frequent causes of Noonan syndrome also the determining factor of the most serious heart defects with high risk of morbidity. **Aim:** The aim of this study is to identify the congenital heart defects associated with PTPN11 mutations in children from our hospital in conjunction with the literature reports.

Materials and method: A number of sixteen pediatric patients, diagnosed with Noonan syndrome were clinically and genetically investigated at Timisoara Genomic Medicine Centre. The molecular testing was performed on MiSeq Illumina platform using Illumina TruSight Cardio Sequencing Panel kit. From sixteen patients' cases for which a mutation was identified, there were twelve in PTPN11 and four in SOS1. In this presentation we focus only on the heterozygous mutations found in PTPN11.

Results: In the remaining twelve cases, pulmonary valve stenosis was found in seven cases; atrial septal defect was identified in 3 cases, aortic coarctation, aortic regurgitation and hypertrophic cardiomyopathy were each identified in two cases, pulmonary regurgitation, heart failure, aortic dysplasia, ventricular septal defect and coronary sinus dilatation were each identified in one case.

Conclusion: The high incidence of cardiac abnormalities suggests that proper cardiac evaluation is important in genetic

counseling, case management, and possibly in the identification of the responsible gene.

Key words: cardiac defects, Noonan syndrome, PTPN 11.

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P05.011.A Limitations in the interpretation of variants of uncertain significance in cardiomyopathies

Clara Herrero-Forte[1,2], *María Fenollar-Cortés*[1,2], *Carmen Cotarelo-Pérez*[1,2], *Victoria Cañadas-Godoy*[3,2], *María Alejandra Restrepo-Córdoba*[4,2], *Laura Daganzo-Sierro*[1,2], *Emma Soengas-Gonda*[1,2], *Raluca Oancea-Ionescu*[1,2]

[1]*Unidad de Genética Clínica. Servicio de Análisis Clínicos. Instituto de Medicina del Laboratorio, Madrid, Spain,* [2]*Instituto de Investigación Clínico San Carlos (IdISSC). Hospital Clínico San Carlos, Madrid, Spain,* [3]*Consulta de Cardiopatías Familiares/Unidad de Arritmias. Servicio de Cardiología., Madrid, Spain,* [4]*Unidad de Insuficiencia Cardiaca y Cardiopatías Familiares. Servicio de Cardiología., Madrid, Spain.*

Introduction: Next Generation Sequencing (NGS) in cardiomyopathies has improved the diagnostic yield. However, detecting variants of uncertain significance (VUS) could be a problem because those with high probability of pathogenicity require additional studies in order to determine their clinical significance.

Materials and Methods: NGS analysis using virtual panel of 50 genes for hypertrophic cardiomyopathy (HCM) or dilated cardiomyopathy (DCM) was performed in 102 patients. Sanger sequencing for cosegregation analysis was able in only 6 of 17 VUS with high probability of pathogenicity. Alpha-galactosidase and lyso-GL-3 levels were measured to confirm Fabry disease. Current scientific evidence was reviewed.

Results: 61 patients with HCM: 15 Pathogenic or likely pathogenic variants (25%) and only 9 VUS were considered with high probability of pathogenicity (15%). 41 patients with DCM: 5 Pathogenic or likely pathogenic variants (12%) and only 8 VUS were considered with high probability of pathogenicity (20%). Cosegregation analysis didn´t allow to confirm the pathogenicity of the 6 VUS with high probability of pathogenicity. An indirect functional study of Fabry disease confirmed the pathogenicity in one case. One VUS with high probability of pathogenicity has been reclassified to likely benign due to a new scientific evidence published by other authors.

Conclusions: Cosegregation studies could be useless in those families with small number of affected relatives. The genetic characteristics of cardiomyopathies, mainly incomplete penetrance, also contributes to a poor performance of the cosegregation studies. Functional studies are more suitable for reclassification of VUS, although they are not usually accessible in healthcare practice.

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P05.012.B *OBSCN* as a candidate gene for apical hypertrophic cardiomyopathy

Busra Unal[1,2], *Nihat Bugra Agaoglu*[1,2], *Ozlem Akgun Dogan*[3,2], *Levent Doganay*[1,2,4], *Mehmet Agirbasli*[5]

[1]*Department of Clinical Genetics, Umraniye Teaching and Research Hospital, University of Health Sciences, Istanbul, Turkey,* [2]*GLAB (Genomic Laboratory), Health Directorate of Istanbul, İstanbul, Turkey,* [3]*Department of Pediatric Genetics, Umraniye Teaching and Research Hospital, University of Health Sciences, Istanbul, Turkey,* [4]*Department of Gastroenterology and Hepatology, Umraniye Teaching and Research Hospital, University of Health Sciences, İstanbul, Turkey,* [5]*Department of Cardiology, Goztepe Teaching and Research Hospital, Istanbul Medeniyet University, Istanbul, Turkey.*

Apical hypertrophic cardiomyopathy(AHCM) involving the distal portion of the left ventricle is a relatively rare form of hypertrophic cardiomyopathy(HCM) with genetic heterogeneity. Recent molecular genetics studies have shown that pathogenic mutations in genes coding sarcomere/Z-band components, including titin/connectin and its associate proteins, plays a role in the etiology. Among these genes, *OBSCN* stands out as a strong candidate and encodes the obscurin, a protein associated with titin/connectin. We present a 58 years old female patient with a likely pathogenic (LP) variant in the *OBSCN* in clinical exome analysis(CES) to elucidate the etiology of AHCM. Next-generation sequencing was performed on the NextSeq500 platform using the virtual panel for Apical hypertrophic cardiomyopathy(AHCM). We reported a heterozygous LP variant on *OBSCN* by attributing PVS1, PM2 criteria. (NM_001098623 GRCh37:hg19: Chr1:228560464 exon 94 c.21989_22002del p. Lys7330Argfs*8).This variation creates a frameshift and causes an amino acid substitution of lysine to arginine at codon 7330(PVS1). This variant is not present in population databases(PM2). There weren`t any other pathogenic or likely pathogenic variants associated with HCM. In their experimental study, Borisov et al. demonstrated that obscurin activity varies and is an important mediator during myocardial hypertrophy. Although this is the first case report in the literature of an LP variation in *OBSCN* in an apical HCM patient, new patients and functional studies are needed to show its association with the disease.

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P05.013.C A novel missense mutation of TNNI3K associated with cardiac conduction disease

Ilyas Ahmad[1,2], *Stephanie Tennstedt*[1,2], *Shafaq Ramzan*[3,1], *Shahid Mahmood Baig*[3], *Jeanette Erdmann*[1,2]

[1]*Institute for Cardiogenetics, University of Luebeck, Luebeck, Germany,* [2]*DZHK (German Centre for Cardiovascular Research) partner site Hamburg / Kiel / Luebeck, Luebeck, Germany,* [3]*National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan.*

Cardiac conduction disease (CCD), which causes altered electrical impulse propagation in the heart, is a serious life-threatening condition with high morbidity and mortality rates. In majority of CCD, the synchronous activity of impulse-generating nodes and impulse-conduction is undermined for the normal heartbeat. CCD exhibit genetic and clinical heterogeneity with diverse underlying pathomechanisms. In this study, we investigated a consanguineous Pakistani family having four affected individuals with heart arrhythmia followed by ventricular septal defect in only one individual. We applied whole exome sequencing (WES) and co-segregation analysis on DNA samples from all available individuals which revealed a novel recessive biallelic mutation (NM_015978.2: c.1531T>C: p.S511P) in the highly conserved kinase domain in cardiac troponin I-interacting kinase (*TNNI3K*) gene. The missense variant was absent from ethnicity matched healthy controls and available public databases. The mutated residue is highly conserved, and its substitution is predicted to be pathogenic by *in silico* prediction methods. Furthermore, molecular dynamics

simulation studies of the dimer suggests that p.S511P has an indirect effect on the orientation of the monomers facing each other, in the direction of a more energetically unfavorable orientation. Since the orientation of the monomers with respect to each other plays a crucial role in the function of the protein, therefore, we hypothesize that *TNNI3K*-S511P might negatively affect the kinase activity of the protein. In conclusion, this study reports a novel recessive mutation p.S511P in *TNNI3K* and expands the spectrum of TNNI3K protein in the pathogenicity of CCD.

Keywords: CCD, TNNI3K

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P05.015.A Diagnostic yield of WES-based gene panels in patients with congenital structural heart defects - a multi-center study

*Marrit M. Hitzert[1], **Rosalie L. Neijzen**[2], Dennis Dooijes[2], Yvonne J. Vos[1], Rosa L. E. van Loon[2], Wilhelmina S. Kerstjens-Frederikse[1]*

[1]University Medical Center Groningen, Groningen, Netherlands, [2]University Medical Center Utrecht, Utrecht, Netherlands.

Introduction: Congenital heart defects (CHD) affect 1% of live births. A monogenetic cause can be identified in 5% to 10% of patients. WES-based gene panels are widely used, while there is no consensus on which genes to include and which patients could benefit most. We evaluated the diagnostic yield of WES-based gene panels in CHD patients.

Patients: We retrospectively evaluated the results from WES-based gene panels in CHD patients in the Netherlands at the University Medical Center Groningen (UMCG) and the University Medical Center Utrecht (UMCU). Inclusion lasted from 2013 (UMCU) and 2017 (UMCG) up until April 2020. We collected clinical data including phenotype and family history of CHD.

Results: A total of 328 patients were included with most patients having a left-sided (n = 117; 35.7%) or conotruncal heart defect (n = 94; 28.7%). In 3/117 and 8/94 patients a (likely) pathogenic variant was demonstrated. Total diagnostic yield was comparable between the UMCG and the UMCU (6.2% and 6.0%, respectively) and was higher in syndromic than non-syndromic patients (12.5% vs. 5%; $P = 0.038$).

Conclusions: Our diagnostic yield of CHD gene panels as used in clinical practice (6%) is comparable to the previously reported yield in study settings (5% to 10%), and is highest in syndromic patients.

Cardiac phenotype	Familial Y/N	Syndromic Y/N	Gene	DNA variant	P/LP
Left atrial isomerism, PAH	N	Y	DNAH11	c.5778+1G>A	P
			DNAI1	c.48+2dupT	P
Right atrial isomerism, right-sided aortic arch, AVSD, (sub)valvular PS	Y	Y	GDF1	c.681C>A, p.(Cys227*)	P
			GDF1	c.681C>A, p.(Cys227*)	P
Dextrocardia, right isomerism, univentricular heart, AVSD, TAPVR	N	Y	PKD1L1	c.2027C>T, p.(Pro676Leu)	LP
			PKD1L1	c.5728C>T, p.(Arg1910Trp)	LP
TGA, VSD	N	N	GDF1	c.681C>A, p.(Cys227*)	P
Truncus arteriosus, VSD	N	N	NKX2-6	c.455dupA, p.(Gln153Alafs*?)	P
			NKX2-6	c.455dupA, p.(Gln153Alafs*?)	P
			PLD1	c.2191A>T, p.(Arg731*)	P
			CEP290	c.133_136delCAAG, p.(Gln45Lysfs*3)	P
Truncus arteriosus, interruption of the aortic arch	N	?	GAT6	c.1417_1426del, p.(Lys473Glyfs*8)	P
Tetralogy of Fallot	N	N	FLT4	c.89C>T, p.(Pro30Leu)	LP
			GDF1	c.681C>A, p.(Cys227*)	P
PS, VSD	Y	Y	EP300	c.3739T>C, p.(Cys1247Arg)	LP
Truncus arteriosus	N	N	CRELD1	c.959delA, p.(Gln320Argfs*25)	LP
Tetralogy of Fallot	N	Y	GLI3	c.642delC, p.(Met215*)	LP
			CRELD1	c.1103T>A, p.(Leu368*)	LP
Truncus arteriosus	N	N	HAND1	c.410_411delinsA, p.(Arg137fs)	P
PS, VSD	N	N	JAG1	c.1278del, p.(Cys427fs)	P
AVSD	Y	Y	MYH11	c.4882A>C, p.(Lys1628Gln)	LP
BAV, TAAD	Y	N	GDF1	c.681C>A, p.(Cys227*)	P
BAV	N	N	FOXC2	c.1402dupG, p.(Glu468Glyfs*?)	LP
HLHS, BAV	Y	Y	PTPN11	c.1505C>T, p.(Ser502Leu)	P
HLHS	N	Y	PTPN11	c.179G>C, p.(Gly60Ala)	LP
ASD	Y	Y	ACTC1	c.904T>A, p.(Ser302Thr)	LP
HLHS, incomplete AVSD, primum septum defect, narrower AoV, hypoplasia aortic arc with COA	Y	Y	PTPN11	c.1529A>C, p.(Gln510Pro)	P
HLHS with mitral valve atresia, hypoplastic aortic arch, PDA ASD	N	N	NOTCH1	c.3177del, p.(Val1060fs)	P

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P05.017.C CHDbase: a genetic knowledge base for congenital heart disease

*Weizhen Zhou[1], Wenke Li[1], Ruby W Wang[2], **Huayan Shen**[1], Wen Chen[1], Qingyi Zeng[1], Hao Wang[1], Meng Yuan[1], Ziyi Zeng[1], Jinhui Cui[1], Fred Y Ye[2], Zhou Zhou[1]*

[1]Chinese Academy of Medical Sciences, Fuwai Hospital, Beijing, China, [2]School of Information Management, Nanjing University, Nanjing, China.

Introduction: Congenital heart disease (CHD) is the most common birth defect, affecting nearly 1 per 100 newborns. CHD covers broad structural heart malformations and shows a complex genetic basis. Yet, the susceptibility genes with different strengths of evidence are scattered in literature without professional data integration and comprehensive analyses. The whole picture of CHD genetics and the genotype-phenotype correlations are unclear.

Method: A total of 1,150 publications were systemically reviewed. Metadata for each study including sample features, experimental design, and statistical results were collected and functional annotations of genes and mutations were performed.

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The patterns of CHD genes across various types were analyzed and a database was constructed.

Result: We integrated 1,139 genes, 1,022 copy number variations and structural variations, 2,641 single-nucleotide variations or small insertions/deletions and 36 linkage regions associated with CHD from 1,150 publications. We extracted a core network of 164 genes using k-core decomposition based on the protein-protein network and revealed the tissue and developmental stage expression patterns, as well as critical biological pathways underlying CHD. Additionally, we refined CHD subtypes using genotype-phenotype correlations and six subtypes were proposed to be more genetically homogeneous. A MySQL-based online database (http://chddb.fwgenetics.org/) was developed to share with the CHD research community.

Conclusion: This genetic database of CHD presented a clear big picture of CHD genetics, and the atlas of CHD genes and subtyping provided important implications for mechanism research and clinical diagnostics and treatment.This work was supported by the CAMS Initiative for Innovative Medicine (2016-I2M-1-016)

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P05.018.D Mutation burden in patients with small unrepaired atrial septal defects

Anne Kathrine Møller Nielsen[1], *Camilla Nyboe*[2], *Anne Sif Lund Ovesen*[2], *Sebastian Udholm*[2], *Malthe Mølgård Larsen*[3], *Vibeke Hjprtdal*[1], *Lars Allan Larsen*[3]

[1]*Rigshopitalet, Copenhagen, Denmark,* [2]*Aarhus University Hospital, Aarhus, Denmark,* [3]*University of Copenhagen, Copenhagen, Denmark.*

Introduction: In a recent nationwide cohort study, we have discovered that patients living with an atrial septal defect (ASD) have a shorter life expectancy, increased risk of atrial fibrillation, pneumonia, and psychiatric issues compared to the general population. The pathophysiological mechanisms are still unknown. The objective of this study is to investigate if this group of patients is burdened by mutations in genes associated with ASD.

Methods: We included 147 patients with an unrepaired ASD. We curated a list of ASD candidate genes, and patients were analyzed for genetic variants in these genes, using targeted next generation sequencing. We filtered for protein altering variants (PAVs) with minor allele frequency (MAF) <0.01 and predicted pathogenicity using the Combined Annotation Dependent Depletion (CADD) method. The number of rare and pathogenic variants in cases were compared with variants identified in 33,370 controls of European ancestry.

Results: We identified 384 rare (MAF < 0.01) PAVs in 59 genes. ASD patients were significantly enriched for very rare (MAF < 0.0001) PAVs compared to controls ($P = 0.0001$). The frequency of PAVs with CADD score ≥ 30 was significantly higher in ASD patients, compared to controls ($P = 0.0042$).

Conclusions: Patients with a small, unrepaired ASD were enriched for rare PAVs within 59 ASD candidate disease genes. Our results suggest that recurrence risk may be increased for this group of patients and warrants further investigations. Funding: This work was supported by Novo Nordic Foundation (Grant no. NNFSA170030576), Brd. Hartmanns Fond, Kong Christian den Tiendes Fond, Dagmar Marshalls Fond and Eva & Henry Frænkels Mindefond.

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P05.019.A Role of xenobiotic biotransformation genes in genetic predisposition of congenital heart diseases

Anna Tsepokina, *Svetlana Shmulevich, Anastasia Ponasenko, Andrey Shabaldin*

Research Institute for Complex Issues of Cardiovascular Diseases, Kemerovo, Russian Federation.

Introduction: Congenital heart diseases are one of the most common multi-factorial fetal abnormalities caused by a complex of endo- and exogenous factors. A number of studies have shown a significant role of genes encoding enzymes involved in different phases of xenobiotic metabolism in the congenital heart diseases pathogenesis.

Material and Methods: In the presented research, 131 children with congenital heart diseases and 101 women having children with this pathology were included in the study group. In control group, 103 healthy children and their mothers were included. Single-nucleotide polymorphisms in the xenobiotic biotransformation genes CYP1A1 (rs1048943), CYP1A2 (rs762551), GSTP1 (rs6591256, rs1871042 and rs17593068) were detected by the real-time polymerase chain reaction. Gene-gene interactions were determined using the Multifactor Dimensionality Reduction method.

Results: The frequency distribution of alleles and genotypes in all studied groups were corresponded to the theoretically expected according to Hardy-Weinberg equilibrium. Comparative analysis of polymorphisms of CYP1A1 (rs1048943), CYP1A2 (rs762551), and GSTP1 (rs6591256, rs1871042 and rs1793068) genes in the study and control groups of women and their children did not show statistically significant differences between groups. We obtained no difference in the frequency of CYP1A1, CYP1A2 and GSTP1 between the study and control groups. At the same time, the genetic combinations GSTP1 (rs6591256)—GSTP1 (rs1871042) and GSTP1 (rs6591256)—GSTP1 (rs1871042)—CYP1A1 (rs1048943) in women; and GSTP1 (rs1793068)—GSTP1 (rs6591256)—GSTP1 (rs1871042)—CYP1A1 (rs1048943)—CYP1A2 (rs762551) in children contribute to the pathogenesis of congenital heart diseases. This study was supported by basic research project №0546-2019-0002.

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P05.020.B Prediction of coronary artery disease: Do risk factor genetic risk scores add value?

Julia Ramírez[1], *Stefan van Duijvenboden*[2], *William J. Young*[1], *Andrew Tinker*[1], *Pier D. Lambiase*[2], *Michele Orini*[2], *Patricia B. Munroe*[1]

[1]*William Harvey Research Institute, London, United Kingdom,* [2]*University College London, London, United Kingdom.*

Introduction: Coronary artery disease (CAD) is heritable and has a polygenic architecture. Current genetic risk scores (GRS) for CAD predict CAD independent from traditional risk factors, however these scores may not capture all genetically pre-determined risk. We hypothesised that a score including additional GRSs for multiple cardiovascular risk factors, explaining additional biology underlying CAD, may improve CAD prediction.

Methods: We used data from 52,254 participants in UK-Biobank without a previous diagnosis of cardiovascular disease. In a training subset (50%, $N = 26,127$), we identified the risk factors significantly associated with CAD using Multivariable Cox regression to build three scores, Sc1 including sex, age and traditional

risk factors, Sc2 including Sc1 and a GRS for CAD, and Sc3 including Sc2, and 26 additional GRSs for traditional and electrocardiogram risk factors.

Results: In an independent test subset (50%, N = 26,127) for CAD, Sc2 had a higher area under the curve (AUC) than Sc1 (0.757 versus 0.735, $P = 7.4 \times 10^{-14}$). The hazard ratios (HRs) (95% confidence interval [CI]) for individuals in the top versus bottom 20% of the distribution were 17.2 (10.7 - 27.7) versus 14.9 (9.3 - 23.7). Adding the two GRSs that remained independently associated with CAD, GRSs for low-density lipoprotein and for triglycerides, did not significantly improve risk stratification (AUC of 0.759, $P = 7.0 \times 10^{-2}$, HR [CI] of 16.6 [10.4 - 26.4]).

Conclusion: Our results do not support additional benefit for CAD prediction by adding several independent GRSs in addition to a CAD GRS alone.

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P05.021.C Clinical impact of re-evaluating genes and variants implicated in dilated cardiomyopathy

*Sophie L.V.M. Stroeks L. V. M. Stroeks[1], Debby M. E. I. Hellebrekers[1], **Ingrid P. C. Krapels**[1], Godelieve R. F. Claes[1], Upsana Tayal[2], Els K. Vanhoutte[1], Artur van den Wijngaard[1], Michiel T. H. M. Henkens[1], James S. Ware[2], Stephane R. B. Heymans[1], Han G. Brunner[1], Job A. J. Verdonschot[1]*

[1]Maastricht University Medical Center, Maastricht, Netherlands, [2]Imperial College London, London, United Kingdom.

Purpose: Accurate interpretation of variants detected in dilated cardiomyopathy (DCM) is crucial for patient care but has proven challenging. We applied a set of proposed refined ACMG/AMP criteria for DCM, re-classified all detected variants in robust genes, and associated these results to patients´ phenotype.

Methods: The study included 902 DCM probands from the Maastricht Cardiomyopathy Registry, who underwent genetic testing. Two gene-panel sizes (extended n = 48; and robust panel n = 14) and two standards of variant classification (standard versus the proposed refined ACMG/AMP criteria) were applied to compare genetic yield.

Results: A pathogenic variant was found in 17.8% of patients, and a variant of uncertain significance (VUS) was found in 32.8% of patients when using method 1 (extended panel (n = 48)+standard ACMG/AMP), compared to respectively 16.9% and 12.9% when using method 2 (robust panel (n = 14)+standard ACMG/AMP), and respectively 14% and 14.5% using method 3 (robust panel (n = 14)+refined ACMG/AMP). Patients with pathogenic variants had significantly lower event-free survival compared to genotype-negative DCM patients.

Conclusion: Stringent gene selection for DCM genetic testing reduced the number of VUSs whilst retaining ability to detect similar pathogenic variants. The number of genes on diagnostic panels should be limited to genes which have the highest signal to noise ratio.

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P05.022.D Experience of an Italian reference laboratory for a rare disease: Hereditary Haemorragic Telangiectasia

Carla Olivieri[1], *Fabio Pagella[2,3], Sara Plumitallo[1], Uroš Hladnik[4], Elisabetta Buscarini[5], Elina Matti[3], Anna Sbalchiero[1], Guido Manfredi[5], Giuseppe Spinozzi[3], Elisabetta De Sando[6], Sara Ugolini[3], Cesare Danesino[1]*

[1]General Biology and Medical Genetics Unit, Department of Molecular Medicine, University of Pavia, Pavia, Italy, [2]Department of Otorhinolaryngology, University of Pavia, Pavia, Italy, [3]Department of Otorhinolaryngology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy, [4]Unità di Genetica Istituto per le malattie rare "Mauro Baschirotto" - B.I.R.D. Foundation o.n.l.u.s., Costozza di Longare (VI), Italy, [5]UOC Gastroenterologia-Centro di riferimento HHT, ASST Ospedale Maggiore di Crema, Crema (CR), Italy, [6]Clinica Pediatrica, Fondazione IRCCS Policlinico San Matteoi, Pavia, Italy.

Introduction: Hereditary Haemorragic Teleangiectasia (HHT) is an autosomal dominant disorder affecting 1:5000-8000 individuals worldwide. Mucocutaneous telangiectases and Arteriovenous malformations in internal organs (mostly lungs, liver and central nervous system) are the disease hallmarks. HHT is caused by pathogenetic variants in *ENG*, *ACVRL1*, *SMAD4* and *GDF2*, belonging to the TGFβ/BMPs pathway. We report the experience of our research laboratory in the last five years (2015-2020), focusing on mutation analysis.

Materials and Methods: Patients' samples were collected by HHT reference centres in Pavia and Crema (CR). Index cases' samples were analysed by NGS sequencing panel of the four HHT causative genes and MLPA; the molecular investigation in patients' relatives was performed by Sanger sequencing.

Results: We collected 334 patients' samples; 99/334 were index cases. Results are summarized in the table below.

	Subjects	ACVRL1	ENG	SMAD4	Not Found	In progress	Unaffected
Index case	99	39 (39.4%)	26 (26.3%)	1 (1%)	27 (27.3%)	6 (6%)	-
Patient's relatives	234	86	60	-	-	8	80
Total patients	333	125	86	1	28	13	80

Conclusions: Our data confirm that HHT is mostly underdiagnosed; however, the presence of a reference center enhances the quality of genetic and clinical results. Moreover, we also corroborate the previous observation that in our country *ACVRL1* is the major HHT gene. Not found subjects can harbor variants in intronic or regulatory regions rather than in novel genes. However, we are collecting WES data to re-analyse these cases. Grant: CO: Italian Ministry of Education, University and Research to the DMM-University of Pavia "Dipartimenti di Eccellenza (2018-2022)"

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P05.023.A Hereditary Haemorrhagic Telangiectasia: evidence of a common ancestor in 19 families from Northern Italy

Anna Sbalchiero[1], *Yasmin Abu Hweij[1], Tommaso Mazza[2], Elisabetta Buscarini[3], Fabio Pagella[4,5], Elina Matti[4], Guido Manfredi[3], Giuseppe Spinozzi[4], Sara Ugolini[4], Carla Olivieri[1]*

[1]General Biology and Medical Genetics Unit, Department of Molecular Medicine, University of Pavia, Pavia, Italy, [2]Unit of Bioinformatics, Fondazione IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo (FG), Italy, [3]UOC Gastroenterologia-Centro di riferimento HHT, ASST Ospedale Maggiore di Crema, Crema (CR),

Italy, [4]Department of Otorhinolaryngology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy, [5]Department of Otorhinolaryngology, University of Pavia, Pavia, Italy.

Introduction: Hereditary Haemorrhagic Telangiectasia (HHT) is an autosomal dominant vascular disorder affecting 1:5000-8000 individuals worldwide. The genes associated with HHT (*ENG*, *ACVRL1*, *SMAD4*, *GDF2*) belong to the TGF-β signalling pathway. Evidence for a "Founder Effect" was demonstrated only few times in the HHT literature. We found 19 HHT unrelated families, coming from the region around Bergamo (Northern Italy) and sharing the same pathogenetic variant: the *ACVRL1 in-frame* deletion c.289_294del (p.H97-N98). Here we suggest the presence of a common ancestor in whom this variant arose and date its origin about 200 years ago.

Materials and Methods: We analysed 88 subjects from 19 families: 66 disease-variant carriers and 22 unaffected. We used eight microsatellite markers spanning 3.7Mb surrounding the *ACVRL1* locus. After haplotype reconstruction, the pathogenetic variant's age estimation was carried out with the DMLE+2.3 software package.

Results: We observed a common disease haplotype in 16/19 families. Three families showed evidence of recombination around the *ACVRL1* locus. Subsequent analyses suggested that the mutation occurred about 8 (95% credible set: 6-11) generations ago, corresponding to about 203 (165-265) years ago.

Conclusions: We hypothesise for the first time a "founder effect" for a HHT pathogenetic variant in Italy. This information is also useful to notify the Bergamo Local Health Authority of a higher incidence of a rare, underdiagnosed disease. This will increase HHT patients' awareness and offer them better clinical care and genetic diagnosis. Grant: CO: Italian Ministry of Education, University and Research to the DMM-University of Pavia "Dipartimenti di Eccellenza (2018-2022)"

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P05.024.B Exosomal microRNA biosignature related with hypertension-associated kidney disease

Ana Ortega[1], *Javier Perez-Hernandez*[2], *Olga Martinez-Arroyo*[1], *Angela L. Riffo-Ramos*[3], *Belen Sanchez-Garcia*[1], *Daniel Perez-Gil*[4], *Fernando Martinez*[5], *Felipe Chaves*[1], *Josep Redon*[1], *Raquel Cortes*[1]

[1]Biomedical Research Institute Clinic Hospital - INCLIVA, Valencia, Spain, [2]INSERM, U1016, I, Cochin Institute, Paris, France, [3]Universidad de La Frontera, Temuco, Chile, [4]Genomics England, Dawson Hall, Charterhouse Square, London, United Kingdom, [5]Medical Research Institute La Fe Hospital, Valencia, Spain.

Introduction: Urinary albumin excretion (UAE) is a marker of cardiovascular risk and renal damage in hypertension. MicroRNAs (miRNAs) packaged into exosomes function as paracrine effectors in cell communication and the kidney is not exempt. This study aimed to state an exosomal miRNA signature related to hypertension-associated kidney disease.

Material and Methods: Exosome samples from patients with hypertension with/without UAE were isolated and characterized. Three unique small RNA libraries from each subject were prepared (total plasma, urinary, and plasma-derived exosomes) for next-generation sequencing profiling. Differentially expressed miRNAs were over-represented in KEGG pathways, and selected miRNAs were validated by RT-qPCR in a confirmation cohort.

Results: A signature of 29 dysregulated circulating miRNAs was identified in UAE hypertensive subjects, regulating 21 pathways. Moreover, changes in the levels of 4 exosomes-miRNAs were validated in a confirmation cohort and found associated with albuminuria. In particular, miR-26a, major regulator of TGF-β signaling, was found downregulated in both type of exosomes when compared with healthy controls and to hypertension normoalbuminurics ($P < 0.01$). Similarly, decreased miR-26a levels were found in podocyte-derived exosomes after TGF-β stress.

Conclusions: Our results revealed an exosomes miRNA signature associated to albuminuria in hypertension. In particular, exosomes miR-26a seemed to play a key role in the regulation of TGF-β, a relevant effector in podocyte damage. These findings support the use of exosomes miRNAs as biomarkers of cardiovascular risk progression and therapeutic tools in early kidney damage. Funding from Carlos III Health Institute (PI12/02615, PI19/01796 PI18/01405, CD18/00166 and with ERDF.

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P05.025.C Identification of a novel *TPM1* variant causing hypertrophic cardiomyopathy in an Indian family

Prabodh Kumar[1], *Ganesh Paramasivam*[2], *Tom Devasia*[2], *Mukund Prabhu*[2], *Maneesh K. Rai*[3], *Prakashini K*[4], *Sandeep Mallya*[5], *Dinesh Reghunathan*[1], *Krishnananda Nayak*[6], *Rajasekhar Moka*[1]

[1]Department of Cell and Molecular Biology, Manipal School of Life Sciences, Manipal Academy of Higher Education, Manipal, India, [2]Department of Cardiology, Kasturba Medical College, Manipal Academy of Higher Education, Manipal, India, [3]Department of Cardiology, Kasturba Medical College, Manipal Academy of Higher Education, Mangalore, India, [4]Department of Radiodiagnosis and Imaging, Kasturba Medical College, Manipal Academy of Higher Education, Manipal, India, [5]Department of Bioinformatics, School of Life Sciences, Manipal Academy of Higher Education, Manipal, India, [6]Department of Cardiovascular Technology, Manipal College of Health Profession, Manipal Academy of Higher Education, Manipal, India.

Hypertrophic cardiomyopathy (HCM) is an inherited cardiovascular disorder characterized by unexplained left ventricular hypertrophy. It affects about 20 million people globally, and its prevalence is estimated to be more than 1 in 500 in the general population. We report a case of an Indian family with clinically defined HCM. Genetic analysis was performed using a targeted amplicon panel containing 28 genes on a semiconductor next-generation sequencer. An autosomal dominant; heterozygous mutation in the *TPM1* gene (α-tropomyosin), NM_001018005.2: c.203A>G, p.Gln68Arg, was identified and validated by Sanger sequencing in all affected members of the family but was absent in the unaffected family member. This variant was not reported in the literature in HCM cases nor described in the general population databases. We did not detect any other known pathogenic or likely pathogenic variants in other genes. α-tropomyosin is an α-helical coiled-coil dimer that spans the actin filament's length as a co-polymer. It plays a critical role in sarcomeric contractile regulation by stabilizing the actin filaments and interacting with other actin-binding proteins. HCM causing *TPM1* mutations act in a dominant-negative, poison polypeptide mechanism, altering this delicate balance and increasing the myofilament's calcium sensitivity causing hypercontraction and hypertrophy. Our finding adds to the mutational spectrum of *TPM1*, which is an uncommon cause of HCM. Identification of novel variants or genes causing HCM can unravel the clinical and genetic heterogeneity in HCM. This work

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P05.026.D Comprehensive genetic study in a young patient with cardiac interventricular septal hypertrophy

Tsenka Boneva[1], *Kalina Belemezova*[2], *Ivan Gruev*[3], *Ivanka Dimova*[4]

[1]*UMHAT Alexandrovska, Cardiology clinic, Sofia, Bulgaria,* [2]*Genetic Laboratory, SAGBAL " Dr Shterev", Sofia, Bulgaria,* [3]*Multidisciplinary Transport Hospital "Tsar Boris III" – Sofia, Sofia, Bulgaria,* [4]*Department of Medical genetics, Medical University Sofia, Sofia, Bulgaria.*

An isolated hypertrophy of the basal segment of the interventricular septum protruding into the outflow tract of the left ventricle may be difficult to distinguish from genetic hypertrophic cardiomyopathy (HCM). Because of potential life-threatening complications associated with a diagnosis of HCM, careful investigations of patients are needed, including genetic analysis. We report here a 16-years old patient, diagnosed with septal hypertrophy (>11 mm thickness) and slight tricuspid valve regurgitation. There was family history for heart-related mortality from both paternal and maternal side. Next generation sequencing was performed to analyze point mutations and deletions/duplications in 125 genes associated with Arrhythmia, Cardiomyopathies and Sudden Cardiac Death. We detected variants of unknown significance (VOUS) with the population frequency of less than 0.1% in 4 genes - listed at the Table below.

Gene	Genetic variant	OMIM disease	Inheritance
BAG3	c.1673C>T (p. Ala558Val)	Dominant dilated cardiomyopathy, Myofibrillary myopathy 6, Charcot-Marie-Tooth type 2	Paternal
DSP	c.5062G>A (p. Ala1688Thr)	Autosomal dominant arrhythmogenic right ventricular cardiomyopathy, dilated cardiomyopathy with sparse hair, keratoderma and agenesis of the teeth, autosomal recessive DC	Maternal
JUP	c.1379G>A (p. Arg460His)	Autosomal dominant arrhythmogenic right ventricular cardiomyopathy and autosomal recessive Naxos disease	Maternal
SCN10A	c.724T>A (p. Ser242Thr)	Autosomal-dominant neuropathy and Brugada syndrome	Paternal

All VOUS were inherited from clinically healthy parents - 2 from the mother and 2 from the father. This points to a probable polygenic etiology of the disease, in which the severity of the disease cannot be accurately determined. Regular assessment of cardiac function is recommended, along with decreasing in high intensity sport activity.

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P05.027.A Molecular genetic testing for hypertrophic and dilated cardiomyopathy in inherited cardiovascular condition genetics service: lessons from a Thai cohort

Objoon Trachoo, *Teerapat Yingchoncharoen, Tawai Ngernsritrakul, Nareenart Iemwimangsa, Bhakbhoom Panthan, Sommon Klumsathien, Sasima Srisukh, Anucha Mukdadilok, Sitthakom Phusanti, Angkana Charoenyingwattana, Takol Chareonsirisutthigul, Wasun Chantratita, Tarinee Tangcharoen*

Faculty of Medicine Ramathibodi Hospital, Bangkok, Thailand.

Introduction: Hypertrophic cardiomyopathy (HCM) and idiopathic dilated cardiomyopathy (DCM) are the most common referral in Inherited Cardiovascular Condition (ICC) Genetics Service. Several issues have to be discussed with patients and families during genetic consultation session, including the option for genetic testing and cardiovascular surveillance in family members.

Materials and Methods: Next-Generation Sequencing data of all patients affected by HCM and idiopathic DCM in ICC clinic were analysed using target gene panel to classify variant pathogenicity. All subjects were asked to contact their asymptomatic first-degree relatives for genetic counselling about their risk and to initiate cardiovascular surveillance.

Results: Sixty subjects (30-HCM and 30-DCM) were enrolled during 1 January 2014 – 31 December 2020. Molecular detection frequency was 53.33% (33.33% pathogenic/likely pathogenic, 20% VUS) for HCM and 20% (10% pathogenic/likely pathogenic, 10% VUS) for DCM. The most prevalent gene attributing to HCM was *MYBPC3* (30%). The others were identified in one of these genes-*ACTN2*, *MYL2*, *MYH7*, *TNNI3*, *TPM1*, *TTR* and *VCL* (3.33% each). Amongst DCM, the variants were detected in *TTN* truncating variant (6.67%), *MYH7* (6.67%), *MYBPC3* (3.33%) and *SCN5A* (3.33%). Following clinical surveillance in family members, the detection frequency of new pre-symptomatic cases was 9.09% for HCM and 7.14% for DCM.

Conclusions: In our cohort, *MYBPC3* was the most prevalent gene related to HCM. Amongst idiopathic DCM, *TTN* and *MYH7* were the most common. Additionally, our genetics service was able to detect new cases approximately 1/10 of asymptomatic family members. **Grants:** Thailand Research Fund and Thailand Centre of Excellence of Life Science.

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P05.028.B If double is the trouble, the triple is undoable: a fatal association of Hypertrophic Cardiomyopathy (*MYH7* pArg719Trp), Heterozygous Familial Hypercholesterolemia (*LDLR* pGlu343Lys) and SARS CoV-2 infection

Nicola Marziliano[1,2], *Alessandro Medoro*[2], *Roberto Ottaviano*[1], *Donatella Mignogna*[2], *Mariano Intrieri*[2], *Giuseppe Giuliani*[1]

[1]*ASST-Rhodense, Rho, Italy,* [2]*University of Molise, Department of Medicine and Health Sciences, Campobasso, Italy.*

Introduction: Hypertrophic Cardiomyopathy (HCM; MIM #192600) and Heterozygous Familial Hypercholesterolemia (HeFH; MIM #144010) are the most common genetic cardiovascular disorders. Both HCM and HeFH can lead to severe heart failure and sudden cardiac death. In this report, we describe a case of a young man who suddenly died after a fatal arrythmia and additionally resulted positive for SARS-CoV-2 virus with high titer in myocardium.

Methods and Results: The proband is a young man (32-year-old) who suddenly died during physical exercise. Autopsy findings showed increased wall thickness of interventricular septum (IVS; 18 mm) and left posterior wall (LPW; 20 mm) with patchy myocardial disarray. Non-obstructive diffuse coronary artery disease (CAD) was also observed. Furthermore, the presence of pulmonary thromboembolism with lymphocytic myocarditis prompted the search of cardiotropic viruses within the myocardium. Real-Time PCR (RT-PCR) tested positive for a high concentration of SARS-CoV-2. Molecular autopsy identified two genetic variants classified as pathogenic in the *MYH7* (p. Arg719Trp) and *LDLR* (p.Glu343Lys) genes. Co-segregation analysis via Sanger sequencing within the family (N = 22) showed that *LDLR* mutation was maternally inherited, while *MYH7* genetic lesion was *de novo*.

Conclusion: Electrical remodeling associated with a genetic substrate and a concomitant presence of diffuse CAD and SARS-CoV-2-induced myocarditis might trigger a fatal arrhythmia. This is of paramount importance for the first- or second-degree relatives in which the identification of the pathogenic substrate, that renders them vulnerable to an increased risk for life-threatening cardiac events, including sudden death, might prompt for clinical and tailored treatments.

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P05.029.C 'Sequencing of cardiomyopathy genes in patients with hypertrophic cardiomyopathy reveals enrichment for rare variants in the genes for arrhythmogenic right ventricular cardiomyopathy'

Ramil Rinatovich Salakhov[1], *Maria V. Golubenko*[1], *Alexey A. Zarubin*[1], *Elena N. Pavlyukova*[2], *Alexander F. Kanev*[2], *Oleg S. Glotov*[3], *Diana A. Alaverdian*[3], *Viktoriia V. Tsay*[3], *Nail R. Valiakhmetov*[1], *Maria S. Nazarenko*[1]

[1]*Research Institute of Medical Genetics, Tomsk, Russian Federation,* [2]*Cardiology Research Institute, Tomsk, Russian Federation,* [3]*City Hospital No. 40, Saint Petersburg, Russian Federation.*

Introduction: Hypertrophic cardiomyopathy (HCM) is caused presumably by mutations in the cardiac sarcomere genes. However, mutations in other genes can be causal, and some modifying loci are suggested. In some genes, mutations can lead to different phenotypes, and more than one mutation can be identified in the patient.

Materials and methods: We studied 46 cardiomyopathy genes with NGS panel "TruSight Cardiomyopathy" (Illumina) in 12 patients with HCM. The effects of the identified variants were assessed according the ACMG guideline.

Results: 6 pathogenic / probably pathogenic variations in the cardiac sarcomere genes (*MYH7, MYBPC3, TNNT2, ACTNA, MYOZ2*) were found, including 3 novel variants. One patient had pathogenic variant in the gene for Danon disease (*LAMP2*). In addition, one patient had premature termination variant in the *PKP2*, and the patient with the *TNNT2* mutation had additional frameshift variant in the *DSC2*. Mutations in these genes cause arrhythmogenic right ventricular cardiomyopathy (ARVC), while our patients had clear HCM phenotype. Moreover, we identified several rare (<1% in GnomAD) nonsynonymous variants which were estimated as benign, likely benign or of uncertain significance. In particular, more than one patient had these rare variants in the *LDB3, DSP, TMEM43* genes which are known as genes for ARVD and/or dilated cardiomyopathy.

Conclusion: Accumulation of rare variants in the ARVC genes has been found in patients with HCM that requires further investigation. These results highlight the idea of genetic continuum for different cardiomyopathy phenotypes.

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P05.030.D MYH7 p.(Arg1712Gln) is a founder pathogenic variant in an international large cohort of hypertrophic cardiomyopathy patients

Luisa Marsili[1,2,3], *Francesco Russo*[2], *Flavie Ader*[4,5,6], *Marie-Line Bichon*[7,8], *Laurence Faivre*[9], *Arjan C. Houweling*[2], *Bertrand Isidor*[7,8], *Ronald H. Lekanne Deprez*[2], *Benoit Mazel*[9], *Sandra Mercier*[7,8], *Gilles Millat*[10,11,12], *Jan G. Post*[13], *Pascale Richard*[4,5,14], *Irma van de Beek*[2], *Alexa M. C. Vermeer*[2], *Ludolf G. Boven*[15], *Jan D. H. Jongbloed*[15], *Peter van Tintelen*[2,13]

[1]*Univ. Lille, Lille, France,* [2]*Department of Clinical Genetics, Amsterdam UMC, University of Amsterdam, Amsterdam, Netherlands,* [3]*CHU Lille, Clinique de Génétique, Lille, France,* [4]*HU Pitié Salpêtrière-Charles-Foix, DMU BioGem, Service de Biochimie Métabolique, UF de cardiogénétique et myogénétique moléculaire et cellullaire, Paris, France,* [5]*Sorbonne Université, UMRS1166 Equipe 1, Paris, France,* [6]*UFR Pharmacie, Faculté de Santé, Université de Paris, Paris, France,* [7]*CHU de Nantes, Service de Génétique Médicale, Nantes, France,* [8]*L'institut du thorax, INSERM, CNRS, UNIV Nantes, Nantes, France,* [9]*Centre de génétique et FHU TRANSLAD, CHU Dijon, Dijon, France,* [10]*Laboratoire de Cardiogénétique Moléculaire, Centre de Biologie et Pathologie Est, Hospices Civils de Lyon, Lyon, France,* [11]*Institut NeuroMyoGène, CNRS UMR 5310, INSERM U1217, Université Claude Bernard Lyon 1, Lyon, France,* [12]*Université de Lyon, Lyon, France,* [13]*Department of Genetics, University of Utrecht, University Medical Center, Utrecht, Netherlands,* [14]*APHP, Centre de référence pour les maladies cardiaques héréditaires, Hôpitaux Universitaires Pitié-Salpêtrière, Paris, France,* [15]*University of Groningen, University Medical Center Groningen, Department of Genetics, Groningen, Netherlands.*

Introduction: The *MYH7* c.5135G>A p.(Arg1712Gln) variant has been identified in several hypertrophic cardiomyopathy (HCM) patients worldwide and it is classified as likely pathogenic on ClinVar. Using data from a large international cohort, we delineate its associated phenotype, gain more evidence for its pathogenicity, and evaluate its founder effect.

Materials and Methods: We retrospectively collected clinical and genetic data of 22 probands and 74 family members. To determine the founder status, haplotypes were reconstructed for 42 patients.

Results: Fifty-three individuals carried the *MYH7* p.(Arg1712Gln) variant, 38 (72%) were diagnosed with HCM. The mean age of HCM diagnosis was 48.8 years (SD 18.1; range 8-74). The clinical presentation ranged from asymptomatic left ventricular hypertrophy to arrhythmias (atrial fibrillation as well as malignant ventricular arrhythmias). Aborted sudden cardiac death (SCD) leading to HCM diagnosis occurred in one proband at the age of

68, and family history of SCD was reported by five (39%) probands. No heart failure death nor heart transplant were reported. Women had a generally later-onset disease with 14% of female carriers diagnosed with HCM at age 50, compared with 54% of male carriers, and penetrance reaching 95% and 92% at age 70 in men and women, respectively. The disease was fully penetrant at age 75 in both sexes. Haplotype analysis showed a founder origin in the majority of patients.

Conclusions: Our data showed that *MYH7* p.(Arg1712Gln) is a pathogenic founder variant in HCM and that cardiac screening should be pursued after the seventh decade in healthy carriers, especially women.

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P05.031.A A new perspective in the study of genetic basis of hypertrophic cardiomyopathy

Elena V. Filatova[1], *Natalia S. Krylova*[2], *Ivan Vlasov*[1], *Maria Maslova*[2], *Natalia Poteshkina*[2], *Petr A. Slominsky*[1], *Maria Shadrina*[1]

[1]*Institute of Molecular Genetics of National Research Centre «Kurchatov Institute», Moscow, Russian Federation,* [2]*Pirogov Russian National Research Medical University, Moscow, Russian Federation.*

Introduction: It is now generally accepted that the exact genetic cause of hypertrophic cardiomyopathy (HCM) is unknown in at least 25% of hereditary cases. To date, a causal relationship with the development of HCM has been established for 10 genes; for another 20 genes, there are data on individual clinical cases indicating such a relationship. However, the full range of pathogenic variants, leading to the development of HCM, has not yet been described. In this regard, the aim of our study was to search for new genes, pathogenic variants in which may be potentially involved in the pathogenesis of HCM.

Materials and Methods: The study included 98 non-related patients with HCM. NGS of exons of 4800 genes associated with the development of various diseases was carried out, and bioinformatic analysis was performed to predict the potential pathogenicity of variants.

Results: The analysis identified 73 potentially pathogenic variants in 43 genes, for which a connection with the development of HCM was not previously shown, but which are involved in the development of other pathologies of the cardiovascular system. Most of the genes identified are involved in the functioning of the heart in general and the sarcomere in particular.

Conclusion: Thus, we were able to identify new variants and genes that may lead to the development of HCM or may be involved in the pathogenesis of the disease. This work was supported by the Russian Foundation for Basic Research (grants no. 19-015-00343) and the Russian Science Foundation (grants no. 21-75-20120).

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P05.032.B The expression level of cytokine genes in the cases of native heart valves in infectious endocarditis

Maxim Sinitsky, Anna Tsepokina, ***Maria Khutornaya****, Anastasia Ponasenko*

Research Institute for Complex Issues of Cardiovascular Diseases, Kemerovo, Russian Federation.

Introduction: Infective endocarditis (IE) is a septic inflammation of the endocardium generally caused by bacteria. Recognition of microbial patterns, cytokine and acute phase responses, hemostasis features and alterations in plasma lipid and calcium profile all have been reported to affect pathogenesis and clinical course of IE.

Material and Methods: The expression level of *IL1B, IL6, IL8, IL10, IL12A, IL12B, IL18, IL23, IL33, CCL2*, and *IL1RL1* has been investigated using biopsies of native mitral, aortic, and tricuspid valves obtained during surgical correction of acquired defect from 25 patients with infectious endocarditis. Biopsies of native mitral and aortic valve cusps from 12 patients who underwent surgical correction of acquired heart disease of non-infectious etiology were used as control. We used quantitative PCR with fluorescent dye SYBR Green for determination of the cytokine gene expression level.

Results: This study revealed that genes could be subdivided into three groups: (i) genes with increased expression (*IL1B*, *IL6* and *IL8*); (ii) genes with reduced expression (*IL33* and *IL1RL1*); (iii) genes with unchanged expression (*IL12A*, *IL18*, *IL23* and *CCL2*). The *IL8* gene expression was characterized by the most pronounced increase (9.83 times versus control), while the *IL1RL1* gene demonstrated the most pronounced decrease in its expression (4.17 times). Expression *IL10* and *IL12B* genes was negligible in all samples. This study was supported by basic research project №0546-2019-0002.

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P05.033.C Diagnostic yield of cardiac gene panel testing in inherited cardiac conditions patients in the Republic of Ireland

Jane L. Murphy[1], *Claire W. Kirk*[1], *Terri P. McVeigh*[2], *Luke Kelly*[3], *Joseph Galvin*[4], *Deirdre Ward*[5], *Terence Prendiville*[6], *Catherine McGorrian*[4], *Margaret Gallagher*[4], *Helen Connaughton*[5], *Sally Ann Lynch*[7]

[1]*University College Dublin, Dublin 4, Ireland,* [2]*The Royal Marsden NHS Foundation Trust, London, United Kingdom,* [3]*Royal College of Surgeons in Ireland, Dublin 2, Ireland,* [4]*Mater Misericordiae University Hospital, Dublin 7, Ireland,* [5]*Tallaght University Hospital, Dublin 24, Ireland,* [6]*Children's Health Ireland at Crumlin, Dublin 12, Ireland,* [7]*Children's Health Ireland at Crumlin, Dublin 24, Ireland.*

Background: Inherited cardiac conditions (ICC), comprising primarily cardiomyopathies and cardiac ion channelopathies, predispose to sudden cardiac death. Aim: Investigate the diagnostic yield from cardiac gene panel testing undertaken in

patients (including molecular autopsy in deceased patients) referred to three national ICC clinics from 2002 to 2020.

Methods: Data was collected by interrogation of departmental databases, family charts, and review of molecular genetic diagnostic reports.

Results: We evaluated molecular genetic results from 835 individuals (461 males, 374 females) from 824 families, including 58 deceased patients who underwent molecular autopsy. Three hundred and fifty patients (42%) carried a single variant; 68 patients (8%) were found to have multiple variants (up to a maximum of four). The diagnostic yield of at least one actionable variant (pathogenic/likely pathogenic) was 28%, while at least one variant of uncertain significance (VUS) was detected in 27% of the cohort. We observed a significant association between female sex and detection of an actionable variant. The yield of actionable variants varied by decade of age, ranging from 0% (≥80 years) to 41% (0-9 years). Actionable variants were most frequent in those undergoing a cardiomyopathy panel (35%) and least frequent in those tested for Brugada syndrome genes (14%). Molecular autopsy yielded an actionable variant in 10% of patients, while 30% of the subcohort carried at least one VUS.

Conclusion: Actionable variants were more likely to be detected in females in our cohort. Despite recent gene curation efforts, the high burden of VUS remains a considerable challenge in ICC management.

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P05.034.D Novel missense variant in KCNA5 gene, p. Gly183Arg, associated to ventricular fibrillation during acute myocardial infarction

Ricardo Pan-Lizcano[1], *Lucia Núñez*[1], *P. Piñón*[2], *X Flores*[2], *G Aldama*[2], *R Calviño*[2], *J M. Vázquez-Rodríguez*[3], *Manuel Hermida-Prieto*[1]

[1]*Cardiology Research Group, Instituto de Investigación Biomédica de A Coruña (INIBIC)-CHUAC-UDC, A Coruña, Spain,* [2]*Department of Cardiology, Complexo Hospitalario Universitario de A Coruna (CHUAC)-INIBIC, A Coruña, Spain,* [3]*Department of Cardiology, Complexo Hospitalario Universitario de A Coruña (CHUAC)-CIBERCV, Instituto de Investigación Biomédica de A Coruña (INIBIC), Universidad de A Coruña (UDC), A Coruña, Spain.*

Introduction: Sudden death (SD) due to ventricular fibrillation (VF) during acute myocardial infarction (AMI) is one of the leading causes of death worldwide. It has been suggested that the risk of SD due to VF in AMI has a multifactorial base, where genetic factors play an important role. It has been proposed that variants cardiac excitability genes could play an important role in VF during AMI.

Methods: Rare genetic variants in 36 genes encoded proteins related to pathways involved were analyzed by NGS in 12 patients with FV during AMI. The visualization of the variant was performed using IGV and the bioinformatic analysis of its possible effect was performed with MutationTester, SNAP2, SIFT2, Polyphen and PhD-SNP.

Results: The variant p.Gly183Arg in *KCNA5* gene was identified in a 68 years old male. The amino acid substitution c.547G>C in *KCNA5* gene produces the variant p.Gly183Arg in the Kv1.5 channel, a voltage-gated potassium channel responsible for the ultrarapid delayed rectifier potassium current. All bioinformatic tools used suggested deleterious function of the protein that present the variant.

Conclusions: Our results suggest that variants in the *KCNA5* gene might be associated with an increased risk of VF in AMI leading to SD, concurring with previous reports. Thus, it is essential to use an ambitious strategy, including all genes related to cardiac excitability, to clarify the pathophysiological basis of VF in AMI.

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P05.035.A Breakpoints of two duplications in the LDL-receptor gene in the Czech Republic

Katerina Konecna, *Lukas Tichy*

Center of Molecular Biology and Genetics, University Hospital Brno, Brno, Czech Republic.

Introduction: Familial hypercholesterolemia (FH) is an autosomal dominant disorder associated with elevated levels of low density lipoprotein cholesterol, leading to increased risk of cardiovascular disease. The most common cause of FH in the Czech Republic (CR) is a mutation in the low density lipoprotein receptor gene (*LDLR*). The *LDLR* gene contains a high number of *Alu* repeats, making it prone to *Alu*-mediated rearrangements. As a result, out of all Czech probands with a *LDLR* mutation, almost 10 % are carriers of a deletion/duplication spanning whole exons. The most common rearrangement of the *LDLR* gene in the CR is a duplication of exons 2-6 (exon2_6dup), which is also the sixth most common *LDLR* mutation in the CR. Duplication of exons 16-18 (exon16_18dup) is the fourth most common rearrangement of the *LDLR* gene in the CR.

Materials and Methods: Sequence surrounding the breakpoint was analyzed by Sanger sequencing in 26 out of 27 known Czech families with exon2_6dup and all 8 known Czech probands carrying exon16_18dup.

Results: All analyzed families with exon2_6dup carried the breakpoint c.67+3545_940+917dup. All known Czech families with exon16_18dup carried the breakpoint c.2312-2067_*1216dup. All breakpoints were located inside an *Alu* element.

Conclusion: Duplications of exons 2-6 and 16-18 of the *LDLR* gene in the Czech population likely arise from one mutation event. It remains to be determined if this is the case for other rearrangements of the *LDLR* gene in the CR. Supported by the Ministry of Health, Czech Republic, grant number NU20-02-00261.

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P05.036.B Genetic profile of left ventricular non-compaction cardiomyopathy - experience of the Polish reference paediatric centre

Dorota Piekutowska-Abramczuk[1], *Agata Paszkowska*[1], *Elżbieta Ciara*[1], *Kamila Frączak*[1], *Małgorzata Rydzanicz*[2], *Agnieszka Pollak*[2], *Piotr Stawiński*[2], *Alicja Mirecka-Rola*[1], *Lukasz Mazurkiewicz*[3], *Marzena Gawlik*[1], *Dorota Siestrzykowska*[1], *Rafał Płoski*[2], *Krystyna Chrzanowska*[1], *Dorota Wicher*[1], *Lidia Ziółkowska*[1]

[1]*Children's Memorial Health Institute, Warsaw, Poland,* [2]*Warsaw Medical University, Warsaw, Poland,* [3]*National Institute of Cardiology, Warsaw, Poland.*

Introduction: Genetically and clinically heterogeneous left ventricular non-compaction (LVNC) is the third most frequent cardiomyopathy in the pediatric population. Clinical manifestation varies from mild to severe symptoms of heart failure, thromboembolic events and arrhythmias. Despite important clinical observations from over the last 25 years, LVNC etiology still remains unknown in 30-50% of cases. We present a series of patients diagnosed with LVNC (mostly isolated at the time of examination) in the 2003-2020 period in the Polish reference paediatric centre.

Materials and Methods: Thirty-two children, mean age 11.2 years were enrolled in this study. Clinical evaluation included: echocardiography, cardiovascular magnetic resonance, NYHA class, ECG, 24-hour Holter ECG and family history. Next-generation sequencing (targeted panel of 25 genes) was performed in all cases.

RESULTS: Pathogenic/likely pathogenic variants in LVNC associated genes were detected in 57% of patients. Recurrent autosomal dominant defects were identified in *HCN4, MYH7, RBM20* and *TTN* genes. Additional defects were detected in single families in: *ACTC1, ACTN2, DES, EYA4, HCCS, KCNQ1, PRDM16* and *TAZ* genes (Barth syndrome). Heart failure, ventricular/supraventricular arrhythmias, third degree atrioventricular block, WPW syndrome and sinus bradycardia were the most common clinical symptoms.

CONCLUSIONS: Genetic evaluation (testing and counseling) is recommended in each patient with isolated or syndromic LVNC. The high genetic yield resulted in explanation of molecular etiology in over half of the studied children and collection of valuable genotype-phenotype data. The study was partially founded by CMHI grant S177/2018.

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P05.037.C Clinical relevance of SCN5A heterozygous mutations in drug-related QT prolongation

Carola Rutigliani[1], *Giuseppe Ciconte*[2], *Emanuele Micaglio*[2], *Michelle Monasky*[2], *Sara Benedetti*[3], *Giorgio Nevio Casari*[3], *Emanuela Teresina Locati*[2], *Carlo Pappone*[2]

[1]*San Raffaele Medical University, Milan, Italy,* [2]*IRCCS Policlinico San Donato, San Donato Milanese, Italy,* [3]*IRCCS San Raffaele Hospital, Milan, Italy.*

Drug - related Q -T trait prolongation is a common problem in several clinical settings: the admnistration of many drugs, ranging from antidepressants to mood modulating drugs, can be responsible for such phenotype, according to current knowledge. In spite of this frequency, most physicians usually do not investigate the molecular mechanism underlying this phenotype but instead just halt administration of the culprit drug upon EKG findings. We describe 4 patients from two unrelated italian families with acute bipolar depression in which Fluoxetine + Olanzapine administration caused Q-T prolongation. Due to Holter ECG results, a genetic testing for suspected Long Q -T syndrome (LQTS) has been performed. In each family a heterozygous *SCN5A* mutation has been identified from peripheral blood extracted genomic DNA. In particular, in the first family the c.5089T>C has been demonstrated, while in the second family the c.655C>T heterzygous mutation has been identified, both without mutations in other LQTS genes. In all patients both drugs have been withdrawn and effectively replaced with Duloxetine in monotherapy; the subsquent clinical workup led to the diagnosis of LQTS in all four patients. These cases suggest the clinical relevance of drug interactions in patient harboring *SCN5A* heterozygous mutations. In such patients, the risk of adverse drug reactions towards common psychiatric drugs might be much higher than in general population, requiring further studies.

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P05.039.A CELSR1 mutations in primary lymphedema

Murat Alpaslan[1], *Sandrine Mestre-Godin*[2], *Isabelle Quere*[2], *Guido Giacalone*[1,3], *Pascal Brouillard*[1], *Miikka Vikkula*[1]

[1]*de Duve Institute, University of Louvain, Brussels, Belgium,* [2]*CHU de Montpellier – Hôpital Saint-Eloi, Montpellier, France,* [3]*Department of Lymphatic Surgery, AZ Sint-Maarten Hospital, Mechelen, Belgium.*

Introduction: Developmental and functional defects in the lymphatic system are responsible for the occurrence of primary lymphedema (PLE). PLE is a chronic debilitating disease caused by increased accumulation of interstitial fluid most commonly in the lower extremities. There is no cure, only symptomatic treatment. A number of genes (n = 29) have been linked to PLE so far, among which CELSR1, in which seven probands and their families have loss-of-function mutations. Previous publications suggest female-limited penetrance.

Materials and Methods: We investigated 755 index patients from our PLE-cohort for variants in CELSR1, using whole-exome sequencing (WES), and performed co-segregation studies for available family members.

Results: We identified 6 mutations predicted to cause loss-of-function (nonsense, frameshift or splice-site alterations) (0.81% of cohort), as well as 30 missense variants predicted to be pathogenic (4.63% of cohort) in CELSR1. All index patients with predicted loss-of-function mutations were female and had PLE on lower extremities. Among all the affected individuals with any of the CELSR1 variants predicted to be pathogenic, 29 were female and 10 were male. Eight females and nine males were asymptomatic carriers of a CELSR1 variant. Two families with CELSR1 mutations causing a premature stop codon were tested on mRNA level without detecting nonsense mediated mRNA decay. Thus, they rather encode a truncated protein.

Conclusions: CELSR1 variants may explain about 1-5% of PLE. Yet, many missense variants need functional validation. Our data underscore the notion that CELSR1 loss-of-function mutations have a higher penetrance in females than in males (83% versus 0% in our series).

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P05.040.B Pathogenic variants affecting the TB5 domain of fibrillin-1 protein in Marfan syndrome and Geleophysic/ Acromicric Dysplasia patients: from tall to short

Pauline Arnaud[1,2,3], *Nadine Hanna*[1,2,3], *Zakaria Mougin*[3], *Geneviève Baujat*[4], *Valérie Drouin-Garraud*[5], *Salima El Chehadeh*[6], *Laurent Gouya*[2], *Sylvie Odent*[7], *Guillaume Jondeau*[2,3,8], *Catherine Boileau*[1,2,3], ***Carine Le Goff***[3]

[1]*AP-HP, Hôpital Bichat, Département de Génétique, Paris, France,* [2]*AP-HP, Hôpital Bichat, CRMR Syndrome de Marfan et pathologies apparentées, Paris, France,* [3]*Université de Paris, LVTS, Inserm U1148, Paris, France,* [4]*AP-HP, Hôpital Necker, Département de Génétique, Paris, France,* [5]*CHU Rouen, Service de Génétique, Rouen, France,*

[6]CHU Strasbourg, Service de Génétique Médicale, Strasbourg, France, [7]CHU Rennes, Service de Génétique clinique, Rennes, France, [8]AP-HP, Hôpital Bichat, Service de Cardiologie, Paris, France.

Introduction: The mostly known fibrillinopathy, Marfan syndrome (MFS), is a multisystem disease with a unique combination of skeletal, cardiovascular and ocular features. The geleophysic/acromicric dysplasia (GD/AD), characterized by short stature, short extremities and joint limitation are described as "the mirror image" of MFS. The numerous *FBN1* mutations identified in MFS are located all along the gene, leading to the same pathogenetic mechanism. Interestingly, in the GD/AD patients, the nineteen heterozygous *FBN1* mutations all affect the TGFβ-binding protein-like domain 5 (TB5).

Material and methods: Between 1996 and 2021, blood samples were obtained for more than 5000 consecutive probands referred nationwide to our laboratory for molecular diagnosis of suspected MFS. The *FBN1* gene was originally screened by bidirectional Sanger sequencing and later by NGS custom capture array.

Results: We identified 5 MFS probands carrying 5 distinct heterozygous mutations affecting the TB5 domain of FBN1. The clinical data for these 5 probands and their 7 relatives showed that all the probands displayed a classical form of MFS, with the involvement of skeletal, cardiovascular, and/or ophthalmological systems. At the molecular level, the variants were 3 missense variants and 2 small in-frame deletions. Strikingly, one missense variant affects an amino acid that was previously involved in GD.

Conclusions: Surprisingly, mutations in the TB5 domain of FBN1 can lead to two opposite phenotypes: GD/AD or MFS suggesting the involvement of tissue specificity mechanism and/or a modifier gene. Further functional studies are ongoing to determine the precise role of this domain in the physiopathology of each disease.

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P05.041.D Unsuspected somatic mosaicism for *FBN1* gene contributes to Marfan syndrome

Pauline Arnaud[1,2,3], *Hélène Morel*[1,4], *Olivier Milleron*[2,3], *Laurent Gouya*[3], *Christine Francannet*[5], *Antoine Da Costa*[6], *Carine Le Goff*[2], *Guillaume Jondeau*[2,3], *Catherine Boileau*[1,2,3], *Nadine Hanna*[1,2]

[1]AP-HP - Hôpital Bichat - Département de Génétique, Paris, France, [2]Université de Paris - LVTS - Inserm U1148, Paris, France, [3]AP-HP - Hôpital Bichat - CRMR syndrome de Marfan et les pathologies apparentées, Paris, France, [4]APHP - Hôpital Saint-Louis - Service de Génétique Moléculaire Neuro-Vasculaire, Paris, France, [5]CHU Clermont-Ferrand - Service de Génétique Médicale, Clermont-Ferrand, France, [6]CHU Saint-Etienne - Service de Cardiologie, Saint-Etienne, France.

Introduction: Individuals with mosaic pathogenic variants in the *FBN1* gene are mainly described in the course of familial screening. In the literature, almost all these mosaic individuals are asymptomatic. In this study, we report the experience of our team on more than 5000 Marfan syndrome (MFS) probands.

Materials and Methods: NGS capture technology allowed us to identify five cases of MFS probands who harbored a mosaic pathogenic variant in the *FBN1* gene.

Results: These 5 sporadic mosaic probands displayed classical features usually seen in Marfan syndrome. Combined with the results of the literature, these rare findings concerned both single nucleotide variants and copy number variations.

Conclusions: This underestimated finding should not be overlooked in the molecular diagnosis of MFS patients and warrants an adaptation of the parameters used in bioinformatics analyses. The five present cases of symptomatic MFS probands harboring a mosaic *FBN1* pathogenic variant reinforce the fact that apparently asymptomatic mosaic parents should have a complete clinical examination and a regular cardiovascular follow-up. We advise that individuals with a typical MFS for whom no single nucleotide pathogenic variant or exons deletion/duplication was identified should be tested by NGS capture panel with an adapted variant calling analysis.

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P05.042.D Genotoxic stress in endotheliocytes is associated with endothelial dysfunction: results of gene expression analysis

Maxim Sinitsky, *Anna Tsepokina, Anton Kutikhin, Daria Shishkova, Anastasia Ponasenko*

Research Institute for Complex Issues of Cardiovascular Diseases, Kemerovo, Russian Federation.

Introduction: It is known that genotoxic stress can induce endothelial dysfunction and atherogenesis. The aim of this research was to study the gene expression signature in endothelial cells exposed to alkylating mutagen mitomycin C (MMC).

Materials and Methods: Primary human coronary- (HCAEC) and internal thoracic artery endothelial cells (HITAEC) exposed to 500 ng/ml MMC (experimental group) and nonexposed control were used in this research. Expression of *DDB1*, *ERCC4*, *ERCC5*, *VCAM1*, *ICAM1*, *PECAM1*, *SELE*, *SELP*, *CDH2*, *CDH5*, *CD34*, *LOX1*, *SCARF1*, *CD36*, *LDLR*, *VLDR*, *NOS3*, *PXDN*, *CAT*, *SOD1*, *SNAI1*, *SNAI2*, *TWIST1*, *GATA4*, *KLF4*, *HEY2*, *ZEB1* genes was evaluated by RT-qPCR immediately after 6 hours of cell incubation with MMC and 24 hours after elimination of MMC from cell cultures.

Results: Immediately after 6 hours of MMC exposure we detected the downregulation of the majority of studied genes excluding *SNAI2* in the experimental group compared to control. After elimination of MMC from the cell cultures the increased expression of leucocyte adhesion (*VCAM1*, *ICAM1*, *SELE*), endothelial-to-mesenchymal transition (*SNAI2*), endothelial mechanotransduction (*KLF4*) genes, and the decreased mRNA level of endothelial differentiation (*PECAM1*, *CDH5*, *CD34*), endothelial-to-mesenchymal transition (*ZEB1*) genes was discovered both in HCAEC and HITAEC. Additionally, HITAEC was characterized by downregulation of oxidative stress (*SOD1*, *PXDN*), endothelial mechanotransduction (*CDH2*) molecules, scavenger receptors (*SCARF1*, *CD36*) and upregulation endothelial-to-mesenchymal transition genes (*SNAI1*, *TWIST1*). In HCAEC, the increased level of scavenger receptors (*LDLR*, *VLDLR*) was detected. This work was supported by a grant from the President of the Russian Federation for young scientists - candidates of science MK-1228.2021.1.4.

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P05.043.A Mutation spectrum in Kazakhstani sudden cardiac death victims revealed by targeted next-generation sequencing of 96 genes associated with cardiac diseases

Ainur R. Akilzhanova[1], *Saule E. Rakhimova*[1], *Ulan A. Kozhamkulov*[1], *Ulykbek Kairov*[1], *Gulbanu Akilzhanova*[2], *Ayaulym Chamoieva*[3], *Tolkyn Z. Zhakupova*[4]

[1]*Center for life sciences, National Laboratory Astana, Nazarbayev University, Nur-Sultan, Kazakhstan,* [2]*Semey Medical University, Pavlodar Branch, Pavlodar, Kazakhstan,* [3]*L. N. Gumilyov Eurasian National University, Nur-Sultan, Kazakhstan,* [4]*Medical University Astana, Nur-Sultan, Kazakhstan.*

Introduction: Sudden cardiac death (SCD) is an unexpected death occurring within the first hour of the onset of symptoms. SCD most commonly occurs in patients with coronary heart disease, whereas inherited cardiomyopathies and primary electrical disorders prevail in younger SCD victims (up to 30% of all SCD in the young). The purpose of the study was to elucidate the mutational spectrum in Kazakhstani SCD victims revealed by next-generation sequencing (NGS) of 96 genes associated with cardiac diseases.

Methods: We screened 37 suspected SCD cases (<50 years) using the customized HaloPlex Target Enrichment System (Agilent) and NGS for 96 genes associated with inherited cardiac syndromes and cardiomyopathies. 27 cases had non-diagnostic structural cardiac abnormalities and 10 cases, diagnosed with a cardiomyopathy post-mortem. ACMG/AMP guidelines were applied for variant interpretation of clinical significance.

Results: 31 rare variants were identified in 17 (63%) of the deceased individuals with non-diagnostic structural cardiac abnormalities. Among them pathogenic variants in KCNQ1, KCNJ2, SCN5A, RYR2 genes were identified. The corresponding frequency in deceased individuals with cardiomyopathies was 35%. The most abundant mutations observed in MYBPC3, LAMA2, MYH6 and GAA.

Conclusion: Genetic screening revealed variants with likely functional effects at high rates, in 63% and 35% of the SCD cases with non-diagnostic and diagnostic cardiac abnormalities, respectively. Targeted NGS screening can support the forensic investigation and help the cardiologist's decision to offer counselling and clinical evaluation to relatives of young SCD victims. Study was supported by a grant from the Ministry Education and Science, Republic of Kazakhstan (AP09563474).

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P05.046.D First case of homozygous variants in *LOX* gene due to a paternal isodisomy in a child with an acute abdominal aortic aneurysm

Carmen Cotarelo-Pérez[1,2], *Raluca Oancea-Ionescu*[1,2], *Clara Herrero-Forte*[1,2], *Jaime Rodríguez-Alarcón*[3,2], *Diego López-de-Lara*[4,2], *Maria Fenollar-Cortés*[1,2]

[1]*Unidad de Genética Clínica, Servicio de Análisis Clínicos, Instituto de Medicina del Laboratorio, Madrid, Spain,* [2]*Hospital Clinico San Carlos, Madrid, Spain,* [3]*Servicio de Cirugía Pediátrica, Instituto del Niño y Adolescente, Madrid, Spain,* [4]*Unidad de Endocrinología Pediátrica, Instituto del Niño y del Adolescente, Madrid, Spain.*

Introduction: Non-syndromic aortic aneurysms are a heterogeneous group of pathologies that present phenotypic variability. Predisposing genetic factors are suspected, although current genetic knowledge of these pathologies is scarce.

Material and Methods: A 14-year-old man underwent surgery for type B aortic dissection due to sporadic and nonsyndromic abdominal aortic aneurysm. The histopathological analysis of the abdominal aortic wall was carried out. Next-Generation Sequencing (NGS) using a panel of 17 genes for aortic aneurysms was performed. Cosegregation studies using Sanger sequencing and microsatellite were necessary for the correct interpretation of NGS results.

Results: No pathogenic or likely pathogenic variants were detected in the NGS. However, NGS revealed a loss of heterozygosity on chromosome 5 that include the *LOX* gene. Sanger sequencing detected a variant of uncertain significance c.773A> T in apparent homozygosity in *LOX* gene.The cosegregation study carried out in his mother did not detect the variant. Although the study of the father was not possible, the study of microsatellites revealed a paternal uniparental isodisomy of chromosome 5. Histopathological analysis showed myxoid degenerative changes of the abdominal aortic tissue.

Conclusions: Pathogenic protein-truncating variants have been widely reported in heterozygosity manner, in adult-onset aneurysms. This is the first case with homozygous missense variant in *LOX* gene who presents an early onset aneurysm, which is uncommon for this gene. Taking into account that the current knowledge about the phenotype-genotype correlation in this pathology is insufficient, the functional study of this variant is necessary for the correct interpretation of these results and genetic counselling.

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P05.047.A Novel TRAF2 variant and KDR deletion are implicated in the pathogenesis of pulmonary arterial hypertension

Natalia Gallego[1], *Shaun Pienkos*[2], *David Condon*[2], *Alejandro Cruz*[3], *Nuria Ochoa*[3], *Julián Nevado*[1], *Pedro Arias*[1], *Stuti Agarwal*[2], *Hiral Patel*[2], *Ananya Chakraborty*[2], *Pablo Lapunzina*[1], *Pilar Escribano*[3], *Vinicio de Jesús*[2], *Jair Tenorio*[1]

[1]*Medical and Molecular Genetics Institute (INGEMM), IdiPaz, Hospital Universitario La Paz, Madrid, Spain,* [2]*Division of Pulmonary and Critical Care Medicine and Department of Medicine, Stanford University, Stanford, CA, USA,* [3]*Pulmonary Hypertension Unit, Department of Cardiology, Hospital Universitario Doce de Octubre, Madrid, Spain.*

Background: Pulmonary arterial hypertension (PAH) is a rare disorder associated with elevation of pulmonary pressures that, if untreated, leads to heart failure and death. Specific genetic variants increase the incidence of PAH.

Methods: Whole exome sequencing (WES) was carried out in a proband with PAH and primary biliary cirrhosis. A custom pipeline for variant prioritization was carried out to obtain candidate variants and Copy Number Variants (CNVs). To determine the impact of *TRAF2* in cell proliferation, we performed an MTS assay on healthy lung pericytes transfected with siRNA specific for *TRAF2*. To measure the effect of loss of *TRAF2* on NF-kappa-beta (NF-κB) activity, we measured levels of Phospho-p65-NF-κB in siRNA-transfected pericytes using western immunoblotting.

Results: WES revealed a *de novo* variant in *TRAF2* and a deletion which encompasses 52 genes, including *KDR*. *TRAF2* encode for immunomodulatory protein that regulate NF-κB activation. The knockdown of *TRAF2* increased NF-κB activity in healthy lung pericytes, which correlated with a significant increase in proliferation. Variants in *KDR* gene has been previously described in PAH associated with interstitial lung disease but as far as we know, no CNVs that include *KDR* has been detected in PAH patients.

Conclusions: We have identified a variant in *TRAF2* and a deletion which include *KDR*. We speculate that loss of function in *TRAF2* promotes pulmonary vascular remodeling by overactivation of the NF-κB signaling activity, however, the deletion can also play a role in the clinical manifestation, suggesting a digenic contribution to the PAH phenotype. Grants: PI18/01233, FCHP unrestricted grant.

N. Gallego: None. **S. Pienkos:** None. **D. Condon:** None. **A. Cruz:** None. **N. Ochoa:** None. **J. Nevado:** None. **P. Arias:** None. **S. Agarwal:** None. **H. Patel:** None. **A. Chakraborty:** None. **P. Lapunzina:** None. **P. Escribano:** None. **V. de Jesús:** None. **J. Tenorio:** None.

P05.048.B *RPL3L* is a novel disease-causing gene in Dilated Cardiomyopathy d Cardiomyopathy

Maria Sabater Molina[1], *Joaquina Maria Pan Perez-Villalobos*[2], *Elisa Nicolas Rocamora*[1], *Mari Carmen Olmo Conesa*[1], *David López Cuenca*[1], *Moises Sorli*[2], *Francisco J. Castro*[2], *Juan Ramon Gimeno Blanes*[1]

[1]*Inherited Cardiac Disease Unit. Universitary Hospital Virgen de la Arrixaca., Murcia, Spain,* [2]*Department of Paediatric Cardiology, Universitary Hospital Virgen de la Arrixaca., Murcia, Spain.*

Background: The genetic cause of dilated cardiomyopathy (DCM) remains unexplained in a substantial proportion of cases. RPL3L encodes the 60S ribosomal protein L3-like protein that is highly expressed in skeletal muscle and heart. Pediatric DCM is a genetic heterogeneous disorder and the yield of the genetic test still remains too low. Our aim was to determine the relation between *RPL3L* mutations and the development of DCM.

Methods: *RPL3L* was sequenced by Sanger technology in 79 DCM probands; all of them with severe DCM. We analyzed the cosegregation of *RPL3L* variants in the family when relatives were available.

Results: We identified RPL3L variants in 8 affected patients (7 (87.5%) females) from 6 families. Frequency was significantly higher in DCM probands (6 of 79 [8.9%]) than in gnomAD and 1000G database. 5 patients were carriers in compound heterocigosis and 4 of them were diagnostic at first year of life and could be studied the cosegregation. The fourth was 64 years and present dimorphic features. The rest of patients with one RPL3L mutation were diagnosed at age from 0 to 9 years.

Conclusions: RPL3L is a novel disease causing gene in DCM accounting for at least 8% of neonatal cases. The *RPL3L* gene should be routinely included in dilated cardiomyopathy genetic testing panels.Table 1. Clinical characteristics in affected carriers of RPL3L variants.

M. Sabater Molina: None. **J. Pan Perez-Villalobos:** None. **E. Nicolas Rocamora:** None. **M. Olmo Conesa:** None. **D. López Cuenca:** None. **M. Sorli:** None. **F.J. Castro:** None. **J. Gimeno Blanes:** None.

P05.049.C Low frequency variants in *STAP1* associated with Familial Hypercholesterolemia

Carmen Rodriguez Jimenez[1], *Javier Sanguino*[1], *Iluminada García-Polo*[2], *J.M. Mostaza*[3], *E. Sevilla*[1], *A. Herranz-Cecilia*[1], *A. Carazo*[1], *N. Gallego*[4], *V. Gómez del Pozo*[5], *L. García-Fernández*[6], *M. Solís*[7], *S. Rodríguez-Nóvoa*[1]

[1]*Metabolic Disease Laboratory, Genetic Department. Hospital Universitario La Paz, Madrid, Spain. Group of dislipemias of genetic origin and metabolic diseases, IdiPAZ, Hospital Universitario La Paz, Madrid, Spain., Madrid, Spain,* [2]*Department of Internal Medicine, Hospital de la Princesa, Madrid, Spain., Madrid, Spain,* [3]*Internal Medicine Service, Hospital Carlos III, HULP, Madrid, Spain., Madrid, Spain,* [4]*Next Generation Sequencing Section. Genetics Department, Hospital Universitario La Paz, IdiPAZ, Madrid, Spain., Madrid, Spain,* [5]*Next Generation Sequencing Section. Genetics Department, Hospital Universitario La Paz, IdiPAZ, Madrid, Spain.of dislipemias of genetic origin and metabolic diseases, IdiPAZ, Hospital Universitario La Paz, Madrid, Spain., Madrid, Spain,* [6]*Preanalytic Section. Genetics Department, Hospital Universitario La Paz, IdiPAZ, Madrid, Spain., Madrid, Spain,* [7]*Bioinformatics Section. Genetics Department, Hospital Universitario La Paz, IdiPAZ, Madrid, Spain. CIBERER, ISCIII, Madrid, Spain., Madrid, Spain.*

Introduction: Autosomal-dominant hypercholesterolemia (FH, OMIM#143890) is a common genetic disorder (1:250-1:500)[1]. Molecular diagnosis can be confirmed by the presence of pathogenic variants in *LDLR* (low density lipoprotein receptor, OMIM#606945), *APOB* (apolipoprotein B, OMIM#107730) and *PCSK9* (proprotein convertase subtilisin/ kexin, OMIM#607786). Recent studies suggested the *STAP1* (signal transducing adaptor family member 1, OMIM#604298) as fourth FH gene[2]. Our objective was to identify variants in STAP1 in FH patients and thereby improve the genetic diagnosis of FH. **Patients and Methods:** The study population included 750 DNA samples from index patients clinically classified as having probable or definitive FH. The samples were analyzed by Next Generation Sequencing (NGS) using a customized panel of 436 genes. Variants of interest were confirmed by Sanger Sequencing. Bioinformatic analysis was performed using algorithms developed by our bioinformatic unit.

Results: Three variants previously reported were found in heterozygous in three patients *in STAP1* two of them with frequency < 0.03 %. The *STAP1:NM_012108.3:c.35G>A*:p.(Arg12His), was conserved, and the in silico predictors showed damage. The *STAP1:NM_012108.3:c.526C>T*:p.(Pro176Ser), was conserved and in silico predictors showed damage. The *STAP1:NM_012108.3:c.120 +6T>C*, did not show impact according to splicing predictors.

Conclusions: There is controversy about the role of STAP1 in FH[3]. Some studies have showed lack of cosegregation in some variants found in *STAP1* in FH patients[4]. In this study we found three variants previously described in patients with hypercholesterolemia. Further studies of cosegregation and functional studies should be performed in order to confirm the role of STAP1 in FH.

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P05.050.D Genetic influences on functional outcome after stroke

Estefanía Alcaide[1], *Nuria Martínez-Gil*[1], *Geòrgia Escaramís*[2], *Uxue Lazcano*[3], *Marina Mola-Caminal*[3], *Caty Carrera*[4], *Cristòfol Vives-Bauza*[5], *Jordi Jiménez-Conde*[3], *Israel Fernández-Cadenas*[4], *Raquel Rabionet*[1]

[1]*Departament de Genètica, Microbiologia i Estadística, Facultat de Biologia, IBUB, IRSJD and CIBERER, University of Barcelona, Barcelona, Spain,* [2]*CIBERESP, Departament de Biomedicina, Facultat de Medicina i Ciències de la Salut, Institut de Neurociències, Universitat de Barcelona, Barcelona, Spain,* [3]*Servei de Neurologia, Hospital Del Mar, Barcelona, Spain,* [4]*Hospital de la Santa Creu i de Sant Pau, Barcelona, Spain,* [5]*Departament de Biologia, Universitat de les Illes Balears, Palma de Mallorca, Spain.*

ID	SEX	AGE AT DIAGNOSIS	PHENOTYPE	TREATMENT	VARIANT 1	VARIANT 2	CURRENT SITUATION
1	Female	2 months	DCM	Pharmacological	R4W	A94T	LVEF 46%
2	Male	2 weeks	DCM	Pharmacological	R161W	G27D	Exitus
3	Female	1.5 months	DCM	Pharmacological	R161W	G27D	Transplanted
4	Female	2 weeks	DCM	PharmacologicalTransplant	R242W	D308N	No transplant rejection
5	Female	65 years	DCM > LVNC Short stature, kyphoscoliosis	Pharmacological	A256T	M168V	LVEF 20-27%
6	Female	9 years	LVNC > DCM	Pharmacological	D308N		LVEF 50%
7	Female	9 years	LVNC > DCM	Pharmacological	D308N		LVEF 58%
8	Female	1 month	DCM > LVNC	Ventricular assist device	c.618C>T		Transplant wait list

Introduction: Stroke is a leading cause of adult disability. There is a large variability in the functional outcome after a stroke, partially regulated by genetic factors. GWAS results highlighted the involvement of common or low-frequency variants in *PP1* and *PATJ*; however, the role of rare variants in stroke recovery remains unsolved.

Materials and Methods: We performed a pilot study analyzing exomes of 90 ischemic stroke cases with extreme functional recovery scores three months after stroke (modified Rankin Scale (mRS) 0-1 vs 4-5), matched by age, gender and stroke severity, to select target genes involved in functional outcome. These targets, as well as selected regions based on previous GWAS results, were included in a capture assay, and sequenced in 700 additional ischemic stroke cases from our hospitals. Rare variants with a CADD score>15 (coding) or a funseq2 score>1.5 (regulatory) were selected for further analysis with a Bayesian-based rare variant association test (BATI) using the discrete mRs scores (0-6, where 6 indicates death) as outcome.

Results: The pilot study highlighted genes involved in angiogenesis, immune system and synaptogenesis, such as *TEK* or *ANGPT2*. Targeted resequencing found association of rare exonic variants in *CNTN5* with worse functional outcome ($p < 0.02$, 100 permutations). Interestingly, the 18 cases carrying *CNTN5* missense variants are highly enriched for mRS = 6 (p-val < 0.0001). One of CNTN5's functions is related to synaptogenesis during nervous system development; nevertheless, further functional experiments are needed to understand how *CNTN5* mutations lead to differences in recovery.

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P05.051.A The value of new and old technologies to decipher a sudden cardiac death case

Georgia Christopoulou[1], Stavroula Samara[1], Aikaterini Oikonomaki[1], Eleni Katsoni[1], Antigoni Miliou[2], Evangelos Oikonomou[2], Charalambos Vlachopoulos[2], ***Pantelis Constantoulakis***[1]

[1]Genotypos MSA, Athens, Greece, [2]1st Department of Cardiology, Hippokration Hospital, National & Kapodistrian University of Athens, Athens, Greece.

Introduction: We present a sudden cardiac death (SCD) case resolved by post mortem genotyping through clinical-laboratory geneticists' collaboration employing new and traditional genomic technologies.

Materials and Methods: A post-mortem negative, blood sample of a clinically healthy, 19-year-old male with no arrhythmogenicity family history was referred for investigation of potential genetic background of SCD. Clinical exome sequencing was performed on DNA extracted from the sample, using Sophia Genetics' Clinical Exome Solution v2. Following preparations according to the manufacturer's protocol, DNA libraries were sequenced on an Illumina NextSeq-500 genetic analyser. Data processing, variant calling and pre-classification were conducted by SOPHiA DDM® bioinformatics pipelines. Multiplex Ligation-dependent Probe Amplification (MLPA) was performed to confirm results and investigate family members.

Results: The lack of any medical history in the proband did not allow targeted genetic investigation, thus a virtual gene panel consisting of 134 genes related to SCD was analyzed for SNVs/Indels/CNVs. Copy number variation analysis revealed a heterozygous *KCNH2* exon 14-15 deletion, further defined and confirmed by MLPA as exon 14-16 deletion. Parental MLPA analysis showed paternal inheritance. Six apparently healthy paternal relatives were tested by MLPA, 3 were found to be deletion carriers.

Conclusions: Mutations in *KCNH2* are related to longQT2 and shortQT1 syndromes and SCD. Although there was a benign medical history, this finding led to retrospective re-evaluation of ECGs where consistent aberrations were recognized. The genomic analysis, not only deciphered the SCD, it led to high-risk family member discovery thus allowing proper counseling and preventive measures in the patients' best interest.

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P05.052.B Sudden death: Bioinformatic analysis of genetic variants

Marisol Delea[1], Guillermo Corró[2], Leonela Luce[3], Monica C. Fabbro[4], Micaela Galain[4], Jorge E. Kolomenski[5], Cecilia S. Fernandez[4], Monica P. Bellazzi[2], Tania Castro[1], Viviana R. Consentino[6], Liliana Francipane[7], Sebastian Menazzi[7], Florencia Giliberto[3], Gustavo Ontiveros[8], Liliana B. Dain[1], ***Carlos D. Bruque***[2]

[1]Centro Nacional de Genética Médica - ANLIS- Malbrán., CABA, Argentina, [2]Hospital de Alta Complejidad- SAMIC - El Calafate, El Calafate, Santa Cruz, Argentina, [3]INIGEM, CONICET / Cátedra de Genética y Biología Molecular, Universidad de Buenos Aires, CABA, Argentina, [4]CEGYR, Medicina Reproductiva y genética, CABA, Argentina, [5]Departamento de Fisiología, Biología Molecular y Celular. Facultad de Ciencias Exactas y Naturales,Universidad de Buenos Aires, CABA, Argentina, [6]Departamento de Neonatología, Hospital Gandulfo, El Calafate, Santa Cruz, Argentina, [7]División de Genética,

Hospital de Clínicas "José de San Martín", Universidad de Buenos Aires, CABA, Argentina, [8]Fundación para la Prevención de la Muerte Súbita, CABA, Argentina.

Introduction: Sudden death in patients over 40 years old is commonly a result of asteroclerotic occlusion of coronary arteries. On the other hand, these events in young patients (<35 years) are usually caused by familial/hereditary genetic diseases (cardiac channelopathy, cardiomyopathies or aortopathy).

Material and Methods: Patients were tested using exome sequencing or targeted gene sequencing. The identified variants were evaluated in molecular homology models generated with the Modeller 9.22 software and stability programs were used to predict ΔΔG caused by residue changes.

Results: Three families with pathogenic variants were reported in this work. The variant (c345G>C) in the ACTA2 gene was found in an index patient with aortic root dilation. The index case of the second family was diagnosed with hypertrophic cardiomyopathy and arrhythmia and bears three different mutations: TNNT2 (c.842A>T), MYBPC3 (c.2429G>A) and SCN5A (c.5408C>G). The last index patient, diagnosed with arrhythmia, carried the variant (c.1308C>A) in the TRPM4 gene. Changes in protein stability and protein surface charges were observed by modelling, suggesting that these mutations may be directly related to the clinical outcomes described for each patient.

Conclusion: Variants were analyzed by means of a global study of genetic variants in several databases, protein structure and protein stability to determine their possible effects and their correlation with patients' phenotypes. The joint analysis of the families and the bioinformatic approach enabled us to determine possible effects for genetic variants.

M. Delea: None. **G. Corró:** None. **L. Luce:** None. **M.C. Fabbro:** None. **M. Galain:** None. **J.E. Kolomenski:** None. **C.S. Fernandez:** None. **M.P. Bellazzi:** None. **T. Castro:** None. **V.R. Consentino:** None. **L. Francipane:** None. **S. Menazzi:** None. **F. Giliberto:** None. **G. Ontiveros:** None. **L.B. Dain:** None. **C.D. Bruque:** None.

P05.053.C Thoracic aortic aneurysms and dissections are associated with *LTBP3* mutations: individual case and literature review

Zhu Guo Yan

PUMC&CAMS Fuwai hospital, Beijing, China.

Normal 0 7.8 磅 0 2 false false false EN-US ZH-CN X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:普通表格; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0cm 5.4pt 0cm 5.4pt; mso-para-margin:0cm; mso-para-margin-bottom:.0001pt; mso-pagination:widow-orphan; font-size:10.0pt; font-family:"Times New Roman",serif;}

Background: With the rapid development of genetic testing technology, more and more HTAAD (Heritable thoracic aortic aneurysm and dissection) -related mutations have been identified. However, many patients with obvious genetic predispositions have still failed to find pathogenic mutations in known genes associated with aortic disease. Hence, we need to update TAAD's list of disease-related genes, which can help to definite causes and pathogenesis of undiagnosed patients and screen the family members of the patients.

Methods: The patient who had no history of hypertension was rehospitalized after surgery for aortic dissection, he presented with aortic root aneurysm and aorta dilates in many parts with no other abnormal signs. The patient was first tested with a negative panel for aortic disease, then we performed the whole exome sequencing (WES)test on the patient, two mutations,c.625dupC (p.Leu209fs) and c.1965delG(p.Arg656fs) in *LTBP3*(latent TGFβ (transforming growth factor β)-binding proteins-3) were found.

Conclusion: We found one case where mutations in *LTBP3* can cause thoracic aortic aneurysm and dissection, which can demonstrate that *LTBP3* is associated with thoracic aortic aneurysm and dissection. It provides a view for the expansion of gene spectrum associated with thoracic aortic aneurysms and dissections, which will be helpful for the clinical diagnosis and clinical intervention.

Z.G. Yan: None.

P05.054.D Telomere length in the pre- and postoperative period of coronary artery disease patients

***Maxim Aidarovich Asanov**, Alena Olegovna Poddubnyak, Anastasia Valerievna Ponasenko*

Research Institute for Complex Issues of Cardiovascular Diseases, Kemerovo, Russian Federation.

Cardiovascular diseases are the leading cause of death worldwide. Decreased or lost function of myocardial cells or blood vessels is the cause of coronary heart disease. Telomeres are located at the ends of chromosomes and consist of tandem repeats TTAGGG. Currently, there are many conflicting research results on the importance of telomere length (TL) in the development of CAD. It is important to assess the role of changes and restoration of leukocyte telomere length in CAD patients before and after surgery. The study included 60 (59 y.o.) patients with CAD and 52 (54 y.o.) healthy people. DNA isolation was carried out using the standard phenol-chloroform extraction method. There was a qPCR for measuring TL. The results of the study showed that the TL in patients with coronary artery disease before surgery and after 5 years statistically significantly differed from the TL of healthy people by 7 times ($p < 0.05$). TL did not differ between patients before surgery and after 5 years of rehabilitation. The effectiveness of measuring telomere length as a marker in the pathology of atherogenesis, in particular ischemic heart disease, is confirmed by the results of the ROC analysis. The area under the ROC-curve AUC $= 0.998 \pm 0.002$. During inflammation, the rate of telomere shortening is accelerated by increased cell division and increased oxidative stress, leading to cellular senescence. This phenomenon contributes to aging of the arteries, which in turn further exacerbates inflammation. Foundation for the Support of Young Scientists in the Field of Biomedical Sciences 2021-1

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P05.055.A Genotypic characterization of an Italian cohort of patients with hereditary transthyretin-related amyloidosis

***Mariabeatrice Sanchini**[1], Marianna Farnè[1], Laura Tonelli[1], Alice Margutti[1], Rachele Rossi[1], Paola Rimessi[1], Marcella Neri[1], Claudio Rapezzi[2,3], Francesca Gualandi[1], Alessandra Ferlini[1]*

[1]Medical Genetics Unit, Department of Medical Sciences, University of Ferrara, Ferrara, Italy, [2]Cardiological Center, S. Anna University Hospital of Ferrara, Ferrara, Italy, [3]Maria Cecilia Hospital, GVM Care & Research, Cotignola, Italy.

Introduction: Hereditary amyloidosis transthyretin-related (hATTR) is a rare, late-onset, autosomal dominant disease due to pathogenic variations, almost invariably missense, in the TTR gene.

Materials and Methods: From 2016 to 2020, hATTR was genetically identified in 95/534 (detection rate 17.8%) patients

coming from Northern and Central Italy Centers. The analyses were performed by Sanger sequencing. The 63.2% (60/95) of patients were males while 36.8% (35/95) were females. In 33.7% (32/95) the analysis was requested as a presymptomatic testing in families with already identified TTR mutations.

Results: We identified 5 known pathogenic/likely pathogenic missense variation types: p.Ile88Leu (77.9%, N = 74), p.Val50Met (12.6%, N = 12), p.Thr139Met (3.2%, N = 3), p.Phe84Leu (3.2%, N = 3), p.Val142Ile (N = 1). We also detected the missense variant p.Val114Leu in one patient, which is novel, though the codon 114 is already known as a site of another missense variation, p.Val114Ala, classified as pathogenic. Many known polymorphisms were also found in 15 out of 95 positive patients, namely p.Gly26Ser (10 patients), p.Thr25Thr (1), c.337-18G>C (2), c.201-31G>A (1), c.70-7C>T (1). Finally, we found a novel intronic variant of uncertain significance c.201-76T>A.

Conclusions: p.Ile88Leu (77.9%) is the most frequent TTR pathogenic variant we have identified and it is mainly associated with a cardiac phenotype. Notably, also the novel variant p.Val114Leu is associated with a hypertrophic cardiomyopathy, underlining the importance of the cardiac phenotype in hATTR. Lastly, we observed an increase requests for TTR genetic testing, which might be related to the approval of the novel orphan drugs (Patisiran and Inotersen).

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P06 Metabolic and Mitochondrial Disorders

P06.001.B Analysis of the phenotype differences in sibs with alkaptonuria

Andrea Zatkova[1], *Andrea Soltysova*[1,2], *Richard Imrich*[1,3], *Helena Glasova*[4], *Birgitta Olsson*[5], *Mohammed Alsbou*[6], *Lakshminarayan Ranganath*[7]

[1]*Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia,* [2]*Department of Molecular Biology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia,* [3]*National Institute of Rheumatic Diseases, Piestany, Slovakia,* [4]*Pharmacology and Clinical Pharmacology, Slovak Medical University, Bratislava, Slovakia,* [5]*Clinical Development, Swedish Orphan Biovitrum, Stockholm, Sweden,* [6]*Faculty of Medicine, Mutah University, Karak, Jordan,* [7]*Department of Clinical Biochemistry and Metabolism, Royal Liverpool University Hospital, Liverpool, United Kingdom.*

Alkaptonuria (AKU) is a rare metabolic disorder caused by mutations within a gene coding for homogentisate 1,2-dioxygenase (*HGD*). Our recent study demonstrated that nitisinone is suitable for treatment of AKU and also allowed collecting of a detailed baseline clinical data for the largest cohort of 139 patients with this rare disease. We performed also the first genotype-phenotype correlation study in this cohort, which showed a small but statistically significant difference in urinary homogentisic acid (HGA) excretion (corrected for dietary protein intake) between variants leading to 1% (G161R) or >30% (M368V, A122V) residual HGD activity. However, there was no difference in serum HGA levels or absolute urinary excretion of HGA, or in the tested clinical symptoms. Taken together, our data indicated that protein intake during the life is more important in respect of the patients phenotype than direct effect of different *HGD* variants on the functionality of HGD protein. In this study, we present analysis of the clinical data focusing on the manifestation of the disease in sib pairs present in the cohort, in order to evaluate phenotypical differences between patients carrying the same genetic variants, thus uncovering other factors influencing severity of the disease. This work was supported by the Slovak National Agency VEGA (grant No. 02/0040/20).

A. Zatkova: None. **A. Soltysova:** None. **R. Imrich:** None. **H. Glasova:** None. **B. Olsson:** None. **M. Alsbou:** None. **L. Ranganath:** None.

P06.003.D Difficulties in diagnosing alpha-mannosidosis

Gabriela Csereoka[1], *Camelia Alkhzouz*[2,1], *Vasilica Plaiasu*[3]

[1]*Children's Emergency Hospital, Cluj-Napoca, Romania,* [2]*"Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania,* [3]*Department of Clinical Genetics, INSMC "Alessandru Rusescu", Bucharest, Romania.*

Introduction: Alpha-mannosidosis is a rare inherited lysosomal storage disorder caused by mutations in the gene encoding for the lysosomal alpha-d-mannosidase, MAN2B1.

Materials and Methods: We report two cases of nonrelative pediatric patients, aged 17 and 4 years, presenting moderate form of alpha-mannosidosis, who were admitted to our department in order to initiate the enzyme replacement therapy. Clinical examination was performed, followed by blood sample collection for biochemical, immunological and hematological analysis and also imagistic examination. The activity of alpha mannosidase, the identification of pathogenic variants in MAN2B1 by next-generation sequencing, and the mannose-rich oligosaccharides urinary level were determined by external laboratories.

Results: Both patients show the main clinical features of the disorder.The younger patient presented normal enzyme activity, a heterozygous pathogenic variant and also a heterozygous variant of uncertain significance, identified in the MAN2B1gene, associating a high level of urinary secretion of mannose -rich oligosaccharides. The older patient has low level of alpha mannosidase activity. Two variants of uncertain significance (homozygous) were identified in MAN2B1 gene.

Conclusions: It is difficult to predict genotype/phenotype relationship in patients affected by alpha- mannosidose. A child's coarse facial features, hearing difficulties, recurrent infections, skeletal abnormalities, affected motor skills and intellectual disability should prompt the physician to investigate the possibility of a lysosomal storage disease, including alpha-mannosidosis. This leads to an earlier diagnosis and initiation of therapy, with the possibility of genetic counseling.

G. Csereoka: None. **C. Alkhzouz:** None. **V. Plaiasu:** None.

P06.004.A Mild forms of hypophosphatasia in Northwest Russia, update

Mikhail Fedyakov, *Y. Eismont, T. Ivaschenko, I. Sosnina, Y. Snegova, S. Scherbak, Y. Barbitoff, A. Shikov, O. Glotov*

City Hospital 40, Saint-Petersburg, Russia, Saint-Petersburg, Russian Federation.

Introduction: Hypophosphatasia (HPP) is a rare heritable metabolic disorder characterized by defective mineralization of bone and/or teeth in the presence of reduced activity of unfractionated serum alkaline phosphatase (ALP). The overall prevalence of severe HPP is range from 1/100 000 to 1/300 000. Mild forms of HPP are more frequent than severe forms - expected prevalence can reach 1/6000 in Western populations. Russian prevalence of mild and severe HPP is still unknown. Genetic analysis provides determining of diagnosis in cases with suspected HPP.

Materials and Methods: We analyzed genomic DNA samples from 259 unrelated individuals with suspected HPP (inclusion criteria: low and/or recurrent low levels of ALP, low growth, recurrent fractures and others). Primers' system for Sanger sequencing was designed and validated for coding 2-12 exons of *ALPL* gene. Exome data of 353 of unrelated individuals (in-house control group) was used for estimation of prevalence in Northwest Russia.

Results: Detection rate was 13,5%: 28 in heterozygous and 7 in compound-heterozygous. 7 novel mutations were detected. Most frequent pathogenic variant was p.E191K in exon 6. The prevalence of this mutation was: 2.9% in suspected HPP group (15/518), 0.28% (2/706 chromosome) in home controls, 0.25% in gnomAD.

Conclusions: Mild forms of HPP predominate in Northwest Russian patients with suspected HPP. Mutation p.E191K in exon 6 is 12 times common in patients with low levels of ALP compare general population.

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P06.005.B Screening of *ASS1* gene in seven families from Lebanon, Syria, Iraq, Kurdistan with citrullinemia type 1 identifies rare and novel variant

Melissa Daou[1], *Mirna Souaid*[1], *Tony Yammine*[1], *Anthony Nemr*[1], *Issam Khneisser*[1], *Nabiha Salem*[1], *Maya Rizkallah*[1], *Manal Mezher*[1], *Adib Moukarzel*[2], *Chantal Farra*[1,3]

[1]*Medical Genetics Unit, Faculty of Medicine, Saint-Joseph University, Beirut, Lebanon,* [2]*Department of Pediatrics, Hotel-Dieu de France University Hospital, Beirut, Lebanon,* [3]*Department of Medical Genetics, Hotel-Dieu de France University Hospital, Beirut, Lebanon.*

Introduction: Citrullinemia is a rare autosomal recessive urea cycle disorder caused by argininosuccinate synthetase (ASS) deficiency. First classified into three types (types I, II, and III) based on biochemical manifestations, it was later reclassified into types I (CTLN1, MIM# 215700) and II (CTLN2, MIM# 603471) based on molecular pathogenesis. It is due to variation in the *ASS1* gene located on chromosome 9q43.11. Incidence of variants differs across populations and ethnic groups. A genotype-phenotype correlation has been established, although not clearly outlined.

Materials and methods: A total of seven families with citrullinemia type 1, four of Lebanese origins, and three others of Syrian, Iraqi, and Kurdish origins were included in our study. Upon informed consent, genomic DNA was isolated from peripheral blood samples and analysis of *ASS1* was carried out.

Results: A novel variant c.286C>A, in exon 5 was described in one Lebanese family with early-onset and severe clinical presentation. Two other homozygous missense variants c.535T>C, in exon 8 and c.787G>A, in exon 10 were identified in two Lebanese families each presenting with late-onset and mild manifestations. In the third Lebanese family, c.535T>C and c.787G>A were both present as heterozygous composite variants. In the Syrian, Iraqi, and Kurdish families, the homozygous c.847G>A, in exon 13 was identified and associated with an early-onset and severe clinical presentation.

Conclusions: A novel variant and three previously reported variants were identified in seven Middle Eastern families, further delineating the molecular basis and genotype-phenotype correlation of citrullinemia type 1.

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P06.006.C a successful treatment with uridine in CAD related disorders

Alaa AlAyed, *Mohammed Almannai*

Section of Medical Genetics, Children's Hospital, King Fahad Medical City, Riyadh, Saudi Arabia.

CAD related developmental and epileptic encephalopathy is an autosomal recessive neurodegenerative disorder caused by mutation in CAD gene that encode a multifunctional enzyme involved in the initial steps of pyrimidine synthesis. This disorder was recently reported, and evidence suggests a positive response to treatment with oral uridine. Exome sequencing in one family identified a homozygous, novel and pathogenic variants in CAD gene in two siblings who presented with developmental regression after seizure onset. In this report we demonstrated a successful treatment with oral uridine in term of mobility, consciousness, communication, and cessation of seizure rendering this disorder as one of the few treatable neurometabolic diseases.

A. AlAyed: None. **M. Almannai:** None.

P06.007.D Clinical, biochemical, and genetic features of patients with congenital disorders of glycosylation in Japan

Nobuhiko Okamoto, *Yoshinao Wada*

Osaka Women's and Children's Hospital, Izumi, Osaka, Japan.

Congenital disorders of glycosylation are heterogeneous diseases caused by defects in various steps in glycosylation pathways. More than 100 genetic defects are known in humans. Many of these defects lead to multi-systemic manifestations, commonly involving the central nervous system. Altered protein glycosylation are classified into N-glycosylation defects, O-glycosylation defects, and combined defects. A type 1 pattern of N-glycosylation disorders (CDG-I) is a glycan assembly defect, a type 2 pattern (CDG-II) is a glycan remodeling defect. Most effective approach to identifying these N-glycosylation disorders is mass spectrometry (MS) using either released glycans, intact glycoproteins or proteolytic peptides as analytes. Among these, matrix-assisted laser desorption/ionization (MALDI) MS of tryptic peptides derived from transferrin can be used to reliably identify signature peptides that are characteristic of CDG-I and II. Additionally, we introduced MS to the O-glycoform profiling of apoCIII. In the present study, MALDI-MS was applied to N- and O-glycosylation disorders. Patients with multisystem disease of unknown etiology from all over Japan were included in this study. The genetic diagnosis was made before or after identifying the glycoform abnormality in this screening. Glycosylation defects were revealed in 50 samples including PMM2-CDG, ALG1-CDG, ALG9-CDG, ALG12-CDG, ALG13-CDG, B4GALT1-CDG, SLC35A2-CDG, ATP6V0A2-CDG, TRAPPC11-CDG, NUS1-CDG, and MAN1B1-CDG. Urinary excretion of Hex4 corresponding to Glc3Man was confirmed in MOGS-CDG. A predominance of PMM2-CDG was detected as in other countries. Some patients with glycoform abnormality have none of the known molecular defects. Further studies with exome analysis are ongoing.

N. Okamoto: None. **Y. Wada:** None.

P06.008.A Genetics and prevalence of Chronic Progressive External Ophtalmoplegia (CPEO) in the Italian region Emilia-Romagna

Leonardo Caporali[1], *Maria Lucia Valentino*[1,2], *Cristina Fonti*[1], *Chiara La Morgia*[1], *Rocco Liguori*[1,2], *Roberto D'Alessandro*[1], *Valerio Carelli*[1,2], *ER-Mito study group*

[1]*IRCCS Institute of Neurological Sciences of Bologna, Bologna, Italy,* [2]*University of Bologna, Bologna, Italy.*

Introduction: Ptosis with or without chronic progressive external ophthalmoplegia (CPEO) is the most common manifestation of mitochondrial myopathy, with maintenance of mitochondrial DNA (mtDNA) defect as disease marker. This defect may lead to qualitative alterations in the form of accumulation of mtDNA multiple deletions in post-mitotic tissues, or quantitative alterations in the form of mtDNA depletion, which may be organ or tissue-specific, secondary to nuclear DNA (nDNA) mutations in genes involved in the replisome machinery, the nucleotide balance and the mitochondrial dynamics.

Materials and Methods: 86 patients with CPEO were recruited: 53 patients were screened for nuclear gene associated with CPEO and 46 for mtDNA rearrangements (single or multiple deletions); 59 skeletal muscle biopsies were available for mtDNA molecular investigations. We evaluated mtDNA deletions by long range PCR and ddPCR, with characterization of deletion breakpoints, and mtDNA copy number by qPCR.

Results: The CPEO prevalence in Emilia-Romagna was 2.32/100.000, reaching 5.07 in Bologna province. Single deletion was found in 37.7% (common deletion in 41.1%), whereas multiple deletions in 8.4%. Genetic defect was detected in 27.9%, being *TWNK* the most frequent gene (44.4%), followed by *POLG* (20.8%), *OPA1* (16.6%), *DNA2* (8.3%), *DGUOK* (4.1%), *MGME1* (4.1%), *RNASEH1* (4.1%), *RRM2B* (4.1%); 6.7 % showed mtDNA depletion, while 30.5% a higher amount, especially in single deletion cases.

Conclusions: Our results provide the first estimates of minimum prevalence of CPEO, revealing single mtDNA deletion and the mtDNA elicase (TWNK) as major genetic cause.Supported by "Programma di ricercar Regione-Università 2010-2012" (PRUa1RI-2012-008)

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P06.009.B Positive association between BGLAP Hind III polymorphism and insulin treatment in type 2 diabetes mellitus

Yelizaveta Harbuzova, *Yaroslav Chumachenko, Olha Obukhova, Viktoriia Harbuzova*

Sumy state university Medical institute, Sumy, Ukraine.

Introduction: According to the current data, bone-derived undercarboxylated protein osteocalcin (OCN) performs the function of a hormone regulating the systemic glucose metabolism. OCN enhances insulin expression and increases its sensitivity in peripheral tissues. Therefore, the aim of the research was to investigate the association between BGLAP HindIII polymorphism and the need for insulin therapy in patients with type 2 diabetes mellitus (T2DM).

Materials and Methods: Venous blood of 153 Ukrainians with diagnosed T2DM was collected for the study. During the treatment, 66 patients (mean age ± SD 63.5 ± 8.03 years) were prescribed insulin preparations and 87 patients (65.55 ± 8.27 years) were observed without insulin. Genotyping was performed using polymerase chain reaction-restriction fragments length polymorphism analysis (PCR-RFLP). All statistical calculations were done in SPSS 22.0 software.

Results: Using binary logistic regression it was found the reduced risk of insulin prescription for C-allele carriers under crude dominant (Pc = 0.016; ORc = 0.423; 95%CI = 0.21-0.85), over-dominant (Pc = 0.006; ORc = 0.335; 95%CI = 0.153-0.736) and additive (Pc = 0.006; ORc = 0.33; 95%CI = 0.149-0.732) models of inheritance. Moreover, the associations remained significant under dominant (Pa = 0.018; ORa = 0.405; 95%CI = 0.192-0.854), over-dominant (Pa = 0.002; ORa = 0.271; 95%CI = 0.117-0.627) and additive (Pa = 0.003; ORa = 0.279; 95%CI = 0.12-0.652) models after the adjustment for age, sex, BMI, smoking, and the presence of arterial hypertension.

Conclusions: It was found that BGLAP HindIII polymorphism is associated with decreased risk of insulin treatment in Ukrainians with T2DM.

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P06.010.C Study of tumor-supressor genes' DNA methylation in patients with type 2 diabetes mellitus

Pavlina Gateva[1], *Ivelina Mihaleva*[2], *Ivanka Dimova*[3]

[1]*Deaprtment of Pharmacology and Toxicology, Medical University - Sofia, Sofia, Bulgaria,* [2]*Department of Pharmacology and Toxicology, Medical University - Sofia, Sofia, Bulgaria,* [3]*Department of Medical Genetics, Medical University - Sofia, Sofia, Bulgaria.*

I**ntroduction**: The epidemiological data represent a significantly increased risk of various cancer forms in patients with diabetes. Type 2 diabetes mellitus (T2DM) and cancer have many common risk factors, but the potential biological link between the two socially significant diseases has not been studied. When examined at the cellular level, both diabetes and cancer are genetic diseases caused by altered gene expression programs. DNA methylation is associated with cancer development. The data are mainly epidemiological and histological, and they do not explain the causes and molecular mechanisms. One possible explanation is the influence of epigenetic modifications in genes, important for oncogenesis.

Materials and methods: We have performed analysis for promoter methylation of 8 tumor suppressor genes (ATM, BRCA1, CDKN1a, Mlh1, Msh2, Rara, Tp53, Xpc) in blood samples of patients with T2DM compared with controls with normal glucose tolerance. Briefly, we used Human Stress & Toxicity PathwayFinder EpiTect Methyl II Signature PCR Array (Qiagen Sciences Inc.).

Results: The highest increase of methylated DNA fraction (by more than 10 times) was detected for promoter methylation of BRCA1 (increase by 18 times), Msh2 gene (increase by 12 times), and CDKN1a (increase by 10 times). The first two genes predispose to breast/ovarian and colon/endometrial cancer.

Conclusion: It is considered that there is a strong link between aberrant methylation of the BRCA1 in white blood cells and breast cancer-related molecular changes that indicate the potential predisposition of BRCA1 dysmethylation for developing breast cancer in diabetic patients.

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P06.011.D Assessing pathogenicity of novel mitochondrial DNA variants

Andrea Winkler[1], *Uwe Ahting*[1], *Bettina Lorenz-Depiereux*[1], *Riccardo Berutti*[1], *Johannes A. Mayr*[2], *Ralf A. Husain*[3], *Andreas Hahn*[4], *Leila Scholle*[5], *Angela Rosenbohm*[6], *Jasmin Lisfeld*[7], *Maja Hempel*[7], *Wolfgang Müller-Felber*[8], *Claudia Catarino*[9], *Tim M. Strom*[1], *Thomas Meitinger*[1], *Thomas Klopstock*[9], *David Thorburn*[10,11,12], *John Christodoulou*[10,11,12], *Holger Prokisch*[1,13], *Saskia Wortmann*[1,2,14], *Matias Wagner*[1,13]

[1]*Institute of Human Genetics, School of Medicine, Technical University München, Munich, Germany,* [2]*Department of Pediatrics, University Hospital Salzburg, Paracelsus Medical University Salzburg, Salzburg, Austria,* [3]*Department of Neuropediatrics, Jena University Hospital, Jena, Germany,* [4]*Department of Child Neurology, University of Giessen, Giessen, Germany,* [5]*Department of Neurology, University of Halle/S., Halle, Germany,* [6]*Department of Neurology, University of Ulm, Ulm, Germany,* [7]*Institute of Human Genetics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany,* [8]*Department of Neuropediatrics, Developmental Neurology and Social Pediatrics, LMU-Campus Innenstadt, University of Munich, Munich, Germany,* [9]*Department of Neurology, Friedrich Baur Institute, University Hospital of the Ludwig- Maximilians-Universität München, Munich, Germany,* [10]*Murdoch Children's Research Institute, Royal Children's Hospital, Victoria, Australia,* [11]*Institute for Molecular Bioscience, The University of Queensland, Queensland, Australia,* [12]*Victorian Clinical Genetics Services, Murdoch Children's Research Institute, Royal Children's Hospital, Victoria, Australia,* [13]*Institute of Neurogenomics, Helmholtz Zentrum München, Munich, Germany,* [14]*Radboud Center for Mitochondrial Medicine, Department of Pediatrics, Amalia Children's Hospital, Radboudumc, Nijmegen, Netherlands.*

Introduction: Diagnostics for suspected mitochondrial diseases can be challenging due to the extremely broad genetic and phenotypic spectrum as well as disease genes on both nuclear and mitochondrial DNA (mtDNA). Whereas most mtDNA variants are well known with undoubted pathogenicity the growing facilitation of next-generation sequencing technologies identifies an increasing number of variants of uncertain significance (VUS) in the mtDNA. In the present study, we aim to assess the pathogenicity of mtDNA variants of uncertain significance.

Materials and Methods: We cumulated mtDNA variants reported as variants of uncertain significance in routine diagnostic exome sequencing and targeted mtDNA next generation sequencing. Clinical data of the individual patients, such as symptoms and MRI abnormalities, were reviewed. An updated scoring system as proposed by Yarham et al. as well as the ACMG criteria were used to assess the variant's pathogenicity.

Results: 36 variants were collected of which 19 are listed as "reported" in MITOMAP and 17 are novel. Of the 19 variants which have been reported before seven could be reclassified to "likely pathogenic", nine to "likely benign" and three remained VUS. Of the 17 novel variants seven were classified as "likely pathogenic", four as "likely benign"and six remained VUS.

Conclusions: We provide evidence for pathogenicity of 14 mtDNA variants and describe six novel variants with potential causal association. The reevaluation of previously collected data provides important evidence for assigning pathogenicity. Collaboratively combining data between institutes allows better understanding of mtDNA variants and is valuable for distinguishing pathogenic from benign variants.

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P06.012.A Urinary extracellular vesicles and their molecular cargo as possible biomarkers of Fabry nephropathy

Tina Levstek[1], *Teo Mlinšek*[1], *Marija Holcar*[1], *Katja Goričar*[1], *Metka Lenassi*[1], *Vita Dolžan*[1], *Bojan Vujkovac*[2], *Katarina Trebušak Podkrajšek*[1,3]

[1]*Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia,* [2]*Centre for Fabry Disease, General Hospital Slovenj Gradec, Slovenj Gradec, Slovenia,* [3]*Clinical Institute for Special Laboratory Diagnostics, University Children's Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia.*

Introduction: Fabry nephropathy (FN) has an important impact on morbidity and mortality in Fabry disease (FD). Current biomarkers are associated with late signs of kidney damage, but they do not predict FN progression. Urinary extracellular vesicles (uEVs) are secreted by cells lining the urinary tract and have not been studied in FD so far. Our aim was to evaluate the association of uEVs and their cargo as possible early biomarkers of FN.

Methods: Small uEVs were isolated by size exclusion chromatography from two urine samples per FD patient ($n = 21$) obtained 5 years apart. We used nanoparticle tracking analysis to determine uEVs size and concentration. We analysed the expression of seven uEVs miRNAs using miRCURY LNA miRNA PCR Assays, two of which served for normalisation.

Results: uEVs concentration, size, and expression of miR-200a-3p, miR-29a-3p, miR-30b-5p, miR-23a-3p, and miR-34a-5p did not differ significantly between patients with and without FN at last follow-up. However, expression of uEVs miR-200a-3p and miR-29a-3p differed significantly between chronological samples ($p = 0.013$ and $p = 0.011$, respectively). These differences were no longer significant among patients without FN. However, when analysing only patients with FN, the concentration of EVs was significantly different ($p = 0.015$) in addition to the above miRNAs ($p = 0.021$ and $p = 0.028$, respectively). In patients with FN, uEVs concentration decreased, while the relative expression of miR-200a-3p and miR-29a-3p increased in the 5-year period.

Conclusion: uEVs miR-200a-3p and miR-29a-3p may represent candidate biomarkers of renal function in FD. Further studies are needed to confirm this association.

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P06.013.B Genetic study of *MTHFR* and *LPA* in patients with familial hypercholesterolemia

Elena Sevilla[1,2], *Carmen Rodríguez-Jiménez*[1,2], *Francisco Arrieta*[3,4,5], *Javier Sanguino*[1,2], *Amanda Herranz-Cecilia*[1,2], *Ana Carazo*[1,2], *Itsaso Losantos-García*[6], *J M. Montejo-Gadea*[7], *V E. Montaño-Fernández*[8], *M E. Rubio-Martín*[8], *Ángela Del Pozo*[9,10], ***Sonia Rodríguez-Nóvoa***[1,2]

[1]*Metabolic Disease Laboratory, Genetic Department. Hospital Universitario La Paz, Madrid, Spain,* [2]*Group of dislipemias of genetic origin and metabolic diseases, IdiPAZ, Hospital Universitario La Paz, Madrid, Spain,* [3]*Department of Endocrinology and Nutrition, Ramón y Cajal University Hospital, Madrid, Spain,* [4]*Ramón y Cajal Health*

Research Institute (IRYCIS), Madrid, Spain, [5]CIBER of Pathophysiology of Obesity and Nutrition (CIBEROBN), Madrid, Spain, [6]Biostatistics Unit. Hospital Universitario La Paz, IdiPAZ, Madrid, Spain, [7]Pre-analytic Section. Genetics Department, Hospital Universitario La Paz, IdiPAZ, Madrid, Spain, [8]Next Generation Sequencing Section. Genetics Department, Hospital Universitario La Paz, IdiPAZ, Madrid, Spain, [9]Bioinformatics Section. Genetics Service, Hospital Universitario La Paz, IdiPAZ, Madrid, Spain, [10]CIBERER, ISCIII, Madrid, Spain.

Introduction: High levels of plasma Lipoprotein A [Lp(a)] and homocysteine are considered cardiovascular disease risk factors[1,2]. In patients with Familial Hypercholesterolemia (FH), increases in Lp(a) and homocysteine levels could contribute to the cumulative burden of risk factors for atherosclerotic-cardiovascular disease[2]. Herein, we analyze relation of Lp(a) and homocysteine levels and genetic polymorphisms in *LPA* and *MTHFR* genes in patients with FH.

Patients and Methods: A total of 212 patients with probable or definitive FH were included. *MTHFR* and *LPA* genes were analyzed by Next Generation Sequence (NGS) using a customized panel of 287 genes.

Results: The genetic analysis showed 22 variants of interest: 5 in *MTHFR* and 17 in *LPA*. Patients with the variant *MTHFR_NM_005957.4:c.665C>T*;p.(Ala222Val) showed higher homocysteine levels compared with patients without it ($p = 0.004$); patients carry *LPA_NM_005577.2:c.4114C>G*:p.(Lys1372Val), *LPA_NM_005577.2:c.4072C>G*:p.(Lys1358Val) showed lower levels of Lp(a) ($p = 0.010$). The variant *LPA_NM_005577.2:c.5673A>G*:p.(Ile1891Met) was observed in six patients with high levels of Lp(a).

Conclusions: In this study we found that *MTHFR* p.(Ala222Val) and *LPA* p.(Lys1372Val) and p.(Lys1358Val) variants were associated with homocysteine and Lp(a) levels in patients with hypercholesterolemia. The study of variants in *MTHFR* and *LPA* could help to better predict the cardiovascular events since homocystinuria and Lp(a) plasma levels have been considered as cardiovascular risk factors.

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P06.014.C Familial Multiple Lipomatosis - analyses of genetic etiology by whole genome sequencing and delineation of the clinical phenotype

Julie Bjerrelund[1,2], *Martin J. Larsen*[1,3], *Henrik D. Schrøder*[4], *Morten Frost*[2], *Moustapha Kassem*[2], *Lilian B. Ousager*[1,3], *Anja L. Frederiksen*[5,6]

[1]Department of Clinical Genetics, Odense University Hospital, Odense, Denmark, [2]Molecular Endocrinology Unit (KMEB), University of Southern Denmark, Odense, Denmark, [3]Department of Clinical Research, Faculty of Health, University of Southern Denmark, Odense, Denmark, [4]Department of Pathology, Odense University Hospital, Odense, Denmark, [5]Department of Clinical Genetics, Aalborg University Hospital, Aalborg, Denmark, [6]Clinical Institute, Aalborg University, Aalborg, Denmark.

Introduction: Familial Multiple Lipomatosis (FML) is a rare condition, with an autosomal dominant pattern of inheritance, characterized by multiple subcutaneous lipomas. However, the genetic background remains to be identified. In this study we i) evaluated the clinical phenotypes including histopathological analyses and biochemical parameters and ii) performed extensive genetic analyses.

Materials and Methods: Five families including 10 subjects with FML and two healthy family members were recruited. A trio- or single based whole genome sequencing (WGS) (Illumina NovaSeq 6000 platform) approach, and a standard karyotyping were undertaken analyzing DNA from peripheral blood. A clinical interview, physical examination and biochemical analyses of the patients, and histopathological analyses of lipomas were performed.

Results: The patients presented clinical features compatible with FML presenting few to several hundred confluent lipomas of 3-4 mm to 14 cm in diameter. Histopathological analyses demonstrated both lipomas and angiolipomas. No patients had diabetes or ischemic heart disease. Biochemical profiles showed marginally elevated levels of lipids in four patients, p-LDL cholesterol: 3.8-4.4 mmol/L (ref: <3 mmol/L). No mutual disease-causing gene was identified however, candidate genes involved in preadipocyte differentiation (*ATF2, CTSB, AKT2)*, adipogenesis (*CDH13)*, tumour suppressor genes (*PRDM2)*, and cell proliferation *(PTPRZ1, TRIM24)*, were identified in single families. In addition, normal karyotype was observed in all probands.

Conclusions: In four families with FML we did not uncover a single mutual genetic background. Ongoing studies, including additional in-vitro studies of adipocyte differentiation and lipoma gene expression, may discover altered signalling pathways and detail the effects of candidate genes.

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P06.016.A Mutational spectrum and functional analysis of the *HGD* gene variants identified in large Russian cohort of patients with alkaptonuria

Igor Bychkov, ***Andrey Nekrasov****, Elena Kamenets, Marina Kurkina, Georgiy Rychkov, Alexandra Ilyushkina, Aleksandra Filatova, Darya Guseva, Galina Baydakova, Aleksandr Cheblokov, Mikhail Skoblov, Ekaterina Zakharova*

Research centre for medical genetics, Moscow, Russian Federation.

Background: Alkaptonuria (AKU) is a very rare genetic disease caused by mutations in the homogentisate 1,2-dioxygenase gene *HGD*. Deficient activity of this enzyme leads to accumulation of homogentisic acid (HGA) and ochronosis, the darkening of tissues. 49 patients from unrelated families were suspected for AKU, based on characteristic clinical and biochemical (the elevated level of urine HGA) symptoms and were referred for genetic testing.

Results: The homozygous and compound heterozygous variants in *HGD* were found in all patients. c.481G>A; p.(Gly161Arg) mutation was found in 45 of 49 patients and comprised 72.4% of identified alleles, which is probably the highest frequency of this mutation worldwide. 9 novel variants were found: 6 missense, 2 splicing and 1 loss of start-codon. The bioinformatic analysis, protein 3D-modeling and molecular dynamics simulation were performed for the missense variants and strongly suggest their pathogenic effect. Rare synonymous c.753C>T (p.Gly251=) variant was found in 3 cases. cDNA analysis and minigene assay demonstrated that c.753C>T is spliceogenic variant, which causes cryptic splice site activation and 23 b.p. frameshifting deletion in vast majority of corresponding mRNA molecules.

Conclusion: The analysis of the largest Russian cohort of AKU patients allowed us to established the peculiar mutational spectrum, characterized by significant prevalence of c.481G>A; p.(Gly161Arg) mutation. The first pathogenic synonymous variant in *HGD* was functionally characterized, which draws the attention

of researchers to this type of mutations. After the detailed functional analysis and application of ACMG guidelines 9 novel *HGD* variants were classified as pathogenic or likely pathogenic.

I. Bychkov: None. **A. Nekrasov:** None. **E. Kamenets:** None. **M. Kurkina:** None. **G. Rychkov:** None. **A. Ilyushkina:** None. **A. Filatova:** None. **D. Guseva:** None. **G. Baydakova:** None. **A. Cheblokov:** None. **M. Skoblov:** None. **E. Zakharova:** None.

P06.017.B Glycogen Storage Disease diagnosis with Clinical Exome Sequencing that has CNV detection capabilities

*Bülent Uyanık[1], Melike Ersoy[2], **Sezin Canbek**[3]*

[1]Bezmialem Vakif University Faculty of Medicine Medical Genetics Department, Istanbul, Turkey, [2]Bakirkoy Dr Sadi Konuk Research and Education Hospital, Istanbul, Turkey, [3]Umraniye Research and Training Hospital Medical Genetics, Fatih, Turkey.

Introduction: Glycogen Storage Diseases arise from an inherited defect in one of the enzymes responsible for forming glycogen or for releasing glucose from glycogen as it is needed by the body during activity and/or between meals. Disruptions in glycogen metabolism usually result in some level of dysfunction in the liver, muscle, heart, kidney and/or brain. Furthermore, the spectrum of symptoms observed is very broad, depending on the affected enzyme. There are around 16 variants of GSD, plus sub-variants, making about 25 in total. A glycogen storage disorder occurs in about one in 20,000 to 25,000 babies. The future of gene therapy appears promising for the GSDs, promising to provide more efficacious therapy for these disorders in the foreseeable future.

Method: We made clinical exome sequencing that contains4493 genes using Illumina NextSeq-500 sequencer with Sophia Genetics Clinical Exome Solution (CES) kit version-2. All single nucleotide variations (SNV) and also copy number variations (CNV) have analyzed by Sophia DDM® Software with filtering Glycogen Storage Diseasesrelated genes.

Results: In 16 patients CES revealed homozygotes SNV and one homozygote exonic deletions. In 3 patients have compound heterozygote SNV. One patient has a hemizygous mutation on X linked inherited PHKA1 gene. 6 patients who have GSD preliminary diagnosis but CES made the clear definitive diagnosis as different: In 4 cases has SNV, remain 2 has homozygous exonic deletions on FBP1and LPIN1 genes.

Conclusion: CES analysis with CNV capability is efficient and can be recommended as first-tier method for GlycogenStorage Disease suspection.

B. Uyanık: None. **M. Ersoy:** None. **S. Canbek:** None.

P06.018.C Novel deletion in *PHKA2* gene in glycogen storage disease type IXa

***Amanda Herranz-Cecilia**[1,2], Carmen Rodríguez-Jiménez[1,2], Carmen Camarena[3], Javier Sanguino[1,2], Rocío Rosas Alonso[4,5], Ana Carazo[1,2], Juan Manuel Montejo-Gadea[6], Ángela Del Pozo[7,8], Sonia Rodríguez Nóvoa[1,2]*

[1]Metabolic Disease Laboratory, Genetic Department, Hospital Universitario La Paz, Madrid, Spain, [2]Group of "Dislipemias of Genetic origin and Metabolic Diseases", IdiPAZ, Hospital Universitario La Paz, Madrid, Spain, [3]Paediatric Hepatology Service, Hospital Universitario La Paz, Madrid, Spain, [4]Pharmacogenetic Unit, Genetic Department, Hospital Universitario La Paz, Madrid, Spain, [5]Experimental Therapies and novel biomarkers in cancer, Instituto de Investigación Sanitaria del Hospital La Paz (IdiPAZ), Madrid, Spain, [6]Preanalytic Section. Genetics Department, Hospital Universitario La Paz, IdiPAZ, Madrid, Spain, [7]Bioinformatics Section. Genetics Department, Hospital Universitario La Paz, IdiPAZ, Madrid, Spain, [8]CIBERER, ISCIII, Madrid, Spain.

Introduction: The Glycogen storage disease (GSD) type IX is due to a deficiency in phosphorylase kinase (PHK, E.C. 2.7.1.38) activity which incidence is 1:100,000 births being responsible for 25% of all GSD cases. It is classified into two types: liver PHK deficiency and muscle PHK deficiency, and is caused by mutations in *PHKA1*, *PHKA2*, *PHKB* and *PHKG2* genes. The liver PHK deficiency is the most common, pathogenic variants in the *PHKA2* gene are responsible for up to 75% of all cases of GSD IX.

Materials and Methods: A 22-month-old male with clinical suspicion of glycogenosis type VI or IX was referred to our laboratory for genetic study of candidate genes by next generation sequencing (NGS). The child presented giant hepatomegaly, increase in transaminases (AST 1740 IU/L [<95] and ALAT 960 IU/L [<35], hypertriglyceridemia, hypoglycemia, liver biopsy with massive deposit of PAS-positive material, special "fat cheeks" phenotype and delayed walking (19 months).

Results: A novel hemizygous deletion extends from exon 1 to 12 of *PHKA2* gene was found by NGS. This deletion is delimited by sanger sequencing, showing a 62.7Kb deletion in X-chromosome (NC_000023.11:g.18947453_19010180del hg:19), which affects exons 1 to 12 of the *PHKA2* gene and extends upstream to the promoter region and the adjacent gene (*ADGRG2*).

Conclusions: The novel deletion identified is the first partial deletion that affects the first exons and promoter region of the *PHKA2* gene. Deletions in *PHKA2* are not frequent, but their study is necessary for the complete characterization of gene variants in patients with glycogenosis.

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P06.019.D Diagnosis of GM1-Gangliosidosis Type II by WES analysis

***Laura Tonelli**[1], Elena Procopio[2], Flavia Tubili[2], Mariabeatrice Sanchini[1], Alessandra Ferlini[1], Alberta Leon[3], Stefania Bigoni[1]*

[1]Medical Genetics Unit, Department of Medical Sciences, University of Ferrara, Ferrara, Italy, [2]Metabolic and Muscular Unit, Clinic of Pediatric Neurology, Meyer Children's Hospital, Florence, Italy, [3]Research & Innovation SRL, Genetic Laboratory, Padua, Italy.

Introduction: GM1-Gangliosidosis is an autosomal recessive sphyngolipidoses due to deficiency of lysosomal enzyme β-galactosidase, encoded by *GLB1*. The disease is characterized by variable degrees of neurodegeneration and skeletal abnormalities. Three clinical forms displaying different severity and variable residual beta-galactosidase activity are known: Type I (infantile), Type II (late infantile/juvenile) and Type III (adult). To date, Miglustat, approved for the treatment of other lysosomal storage disorders, is used as off-label drug in GM1-Type II and it is the only treatment known to stabilize/slow down the neurological progression.

Materials and Methods: The proband was a 6 year-old female with a normal psychomotor development until age 3, when she began to show language regression, slight impairment of eye contact and motor skills. Biochemical/hematological tests, ophthalmologic evaluation, abdominal ultrasound examination and brain MRI were all normal. *FMR1* and *MECP2* molecular analysis, Array-CGH analysis were all negative. The presence of a psychomotor regression prompted us to proceed with WES (HiSeq

2500 Illumina) for intellectual disabilities in silico gene panel testing.

Results: A *GLB1* compound heterozygous genotype for the missense pathogenic variants c.152T>A (p.I51N), inherited from her mother, and c.602G>A (p.R201H), inherited from her father, was detected. Both variants were already reported in patients with GM1-Type II, leading to 3-5% of residual enzyme activity. The proband was referred to the specific Center for drug administration options.

Conclusions: WES allowed an early diagnosis of GM1-Gangliosidosis which is compulsory for clinical trial enrollment and for allowing therapeutic approach as the off-label use of Miglustat.

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P06.020.A Recurrent hydrops fetalis, a long journey to diagnosis

Sana Skouri[1], *Ines Ouertani*[1], *Soumeya Bekri*[2], *Catherine Caillaud*[3], *Ridha M'rad*[1]

[1]*Departement of Medical Genetics, Charles Nicolle Hopistal, Tunis, Tunisia,* [2]*Department of Metabolic Biochemistry, Rouen University Hospital, Rouen, France,* [3]*Biochemical, Metabolomic, and Proteomic Department, Necker University Hospital Group, Paris, France.*

Introduction: Hydrops fetalis affects 1/1700 to 1/3000 pregnancies. It is mainly a non-immune hydrops fetalis (NIHF). Inborn errors of metabolism account for 1.3% of affected individuals. Lysosomal storage are incriminated in up to 29.6% of NIHF cases. We report the history of a family with recurrent hydrops fetalis revealing a mucopolysaccharidosis type VII (MPS VII).

Materials and Methods: The performed analysis were: electrophoresis of glycoaminoglycans in amniotic fluid, ß-D-glucuronidase activity in chorionic villus and *GUSB* gene sequencing in chorionic villus samples and in maternal blood.

Results: This is the case of a consanguineous couple with the history of an unclassified NIHF.

A first prenatal diagnosis was performed for a second case of fetalis hydrops. The glycoaminoglycans profile in the amniotic liquid was abnormal, though not specific of a particular MPS. The fetopathological examination was strongly suggestive of MPS VII. An increased nuchal translucency motivated a second prenatal diagnosis, showing an accumulation of dermatan sulfate and chondroitin sulfate. The combination of these findings supported the hypothesis of an underlying MPS VII. This diagnosis was finally confirmed, in another case of NIHF, by the absence of beta-D-glucuronidase activity in the amniotic fluid.

For the last pregnancy, molecular analysis revealed a novel variant in the exon 7 of the *GUSB* gene, c.1157A>G (p.Tyr386Cys) predicted to be deleterious.

Conclusion: This case highlights how challenging investigating NIHF etiology could be. Accurate diagnosis of lysosomal storage disorders like MPS VII is essential to give the family an adequate genetic counselling.

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P06.021.B Mutations in *MCAT* cause a nuclear LHON-like optic neuropathy

Sylvie Gerber[1], *Christophe Orssaud*[2], *Josseline Kaplan*[1], ***Jean-Michel Institut Imagine Rozet***[1]

[1]*Laboratory of Genetics in Ophthalmology (LGO), INSERM UMR1163, Institute of Genetic Diseases, Imagine and Paris Descartes University, 75015 Paris, France., PARIS, France,* [2]*Unité Ophtalmologie, Hôpital Européen Georges-Pompidou HEGP, 75015 Paris, France & Centre de Référence des Maladies Rares en Ophtalmologie (OPHTARA). Service d'ophtalmologie, Hôpital Necker Enfants Malades, 75015 Paris, France., PARIS, France.*

Pathologic variants in the malonyl-CoA-acyl carrier protein transacylase (*MCAT*) a nuclear gene encoding a mitochondrial protein involved in fatty acid biogenesis have been reported in a unique family from China including two siblings affected with an insidious optic nerve degeneration in childhood, leading to blindness in the first decade of life. Here, analyzing 51 families with negative molecular diagnosis tests from a cohort of 200 families with hereditary optic neuropathy (HON), we identified two novel *MCAT* mutations in a female patient who presented with acute, sudden, bilateral, yet asymmetric, central visual loss at the age of 20-years. This phenotype is reminiscent of the maternally-inherited Leber hereditary optic neuropathy (LHON), the existence of which has been described only very recently along with causative variants in *NDUFS2* and *DNAJC30*, respectively. Our finding expands the phenotypic presentation of MCAT mutations and the genetic heterogeneity of nuclear LHON-like phenotypes. Although *MCAT* pathologic variants are very uncommon, this gene should be investigated in HON patients, irrespective of the disease presentation.

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P06.022.C Biallelic variants in *SPART* cause a mitochondrial dysfunction and cell cycle arrest in Troyer Syndrome

Chiara Diquigiovanni[1], *Antje Kampmeier*[2], *Elisabetta Cuna*[3], *Nicola Rizzardi*[3], *Irene Liparulo*[3], *Francesca Bianco*[4], *T. G. Haack*[5], *M. Bertrand*[5], *K Khuller*[2], *A. Kuechler*[2], *Christian Bergamini*[3], *Elena Bonora*[4]

[1]*University of Bologna, Department of Medical and Surgical Sciences, DIMEC, Bologna, Italy,* [2]*Institut für Humangenetik, Essen, Germany,* [3]*Department of Pharmacy and Biotechnology (FaBit), Bologna, Italy,* [4]*Department of Medical and Surgical Sciences, DIMEC, Bologna, Italy,* [5]*Institut für Medizinische Genetik und Angewandte Genomik, Tübingen, Germany.*

Mutations in *SPART* (OMIM *607111) cause Troyer syndrome (OMIM #275900), a recessive form of spastic paraplegia resulting in lower extremity spasticity and weakness, degeneration of corticospinal tract axons, short stature, and cognitive defects. *SPART* encodes for Spartin, a multifunctional protein consisting of an N-terminal domain, interacting with microtubules for protein trafficking, and a C-terminal senescence domain. We previously found that a loss-of-function mutation in *SPART* caused mitochondrial dysfunctions characterized by Complex I impairment and altered pyruvate metabolism. Performing whole-exome sequencing two novel compound heterozygous missense variants were identified in *SPART*, in a patient presenting muscle weakness and short stature. The patient's fibroblasts showed an altered mitochondrial network, decreased OXPHOS activity, increased mitochondrial ROS production and mitochondrial membrane potential, vs. control fibroblasts. Consistent with Complex I impairment, an increase in NADH levels, extracellular pyruvate and glycolytic metabolism was observed in mutant cells. Since Spartin interacts with GRP75, modulating the import of mitochondrial proteins, we performed co-immunoprecipitation assay in the patient's fibroblasts, and found that there was no interaction

between GRP75 and the mutant SPART. Immunofluorescence staining in control and patient-derived fibroblasts revealed also a marked nuclear localization of Spartin in the mutant cells, whereas in controls it was evenly distributed in the cells. Noticeably, cell cycle analysis revealed that patient's fibroblasts were retained in S phase. In summary, we report that biallelic missense variants in *SPART* lead to a different cellular distribution of the protein, might alter import and assembly of nuclear-encoded mitochondrial proteins, and converge in a severe mitochondrial dysfunction.

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P06.023.D <Functional analysis of novel *HNF1A* variants found in MODY patients in Slovakia>

Terezia Valkovicova[1], *Zuzana Dobiasova*[1], *Juraj Stanik*[2,1], *Martina Skopkova*[1], *Daniela Gasperikova*[1]

[1]*Slovak Academy of Sciences, Bratislava, Slovakia,* [2]*2nd Department of Pediatrics, National Institute of Children's Diseases and Faculty of Medicine, Comenius University, Bratislava, Slovakia.*

HNF1A-MODY is a type of monogenic diabetes caused by heterozygous pathogenic mutations in the transcription factor HNF1α. Due to the increased number of novel variants in the *HNF1A* gene, the functional characterisation of variants on protein level is necessary for better prediction of their pathogenicity. We performed functional analysis of 6 novel variants and 2 variants previously described as VUS identified in 9 Slovak families. Transactivation activity using luciferase assay, DNA-binding using EMSA, and nuclear localisation using immunofluorescence of mutated HNF1α were compared with the wild-type HNF1α and a set of positive and negative controls. Four variants (p. Tyr163Asn, p. Pro224Leu, p. Leu232Pro, and p. Asn270Ser) located in the DNA binding domain (DBD) of HNF1α revealed significantly reduced < 40% transactivation activity of the WT-HNF1α. One DBD-variant (p. Asn140Asp) had activity decreased only to ~61% and all 3 tested variants located in the transactivation domain (p. His469Tyr, p. His483Arg, p. Gln541His) retained more than 80% activity compared to the WT-HNF1α. Three DBD-variants had decreased DNA binding ability (<40% of the WT-HNF1α), only the p. Pro224Leu variant had ~100% binding. Nuclear localisation was not significantly altered in any of examined mutated proteins. We have confirmed the pathogenicity of four novel *HNF1A* variants located in the region encoding HNF1α-DBD domain using functional studies. In three cases, decreased transactivation activity could be explained by decreased DNA binding. None of the tested variants located in the transactivation domain were found to have effect on the HNF1α activity. Supported by: VEGA 0211/18, VEGA 0131/21

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P06.024.A Functional characterization of 3'UTR *LDLR* variants in Familial Hypercholesterolemia

Javier Sanguino[1,2], *Carmen Rodríguez-Jiménez*[1,3], *Jose María Mostaza*[4], *Elena Sevilla*[1,2], *Amanda Herranz-Cecilia*[1,2], *Ana Carazo*[1,2], *Olga Pernía*[5,6], *Carlos Rodríguez-Antolín*[5,6], *Inmaculada Ibáñez*[5,6], *Sonia Rodríguez-Nóvoa*[1,2]

[1]*Metabolic Disease Laboratory, Genetic Department. Hospital Universitario La Paz, Madrid, Spain,* [2]*Group of dislipemias of genetic origin and metabolic diseases, IdiPAZ, Hospital Universitario La Paz, Madrid, Spain,* [3]*Group of dislipemias of genetic origin and metabolic diseases, IdiPAZ, Hospital Universitario La Paz, Madrid, Spain,* [4]*Internal Medicine Service, Hospital Carlos III, HULP, Madrid, Spain,* [5]*Epigenetics laboratory, INGEMM, Hospital Universitario La PAZ, Madrid, Spain,* [6]*Experimental Therapies and novel biomarkers in cancer, Instituto de Investigación Sanitaria del Hospital La Paz (IdiPAZ), Madrid, Spain.*

Introduction: Familial hypercholesterolemia (FH;MIM#143890) is mainly caused by pathogenic variants in *LDLR* gene (>90% of cases[1]) and variants in *APOB*, *PCSK9* and *LDLRAP1* (10% of cases). However, variants in these genes are only found in 60-80% of patients with a definitive diagnosis of FH[2]. Most of variants are located in exonic regions. In this study, we selected and performed in vitro characterization of variants in non-coding region 3'UTR of *LDLR*, a non-canonical region not screened in routine and that could contribute to genetic diagnostic.

Materials and methods: Analysis of the region 3´UTR *LDLR* of patients remitted to our center was performed by NGS using a customized panel of 198 genes. Variants with population frequency less 0,5%, were selected for in vitro characterization. The 3'UTR *LDLR* variants were generate into the expression vector *LDLR*_NM_000527-Human-cDNA-luciferase reporter by site-directed-mutagenesis and transfected in cell line HepG2. 3'UTR *LDLR* expression was quantified by luminescence assay[3].

Results: Five 3'UTR *LDLR* variants were selected for characterization. The variant at *LDLR*, *c.*653G>C* showed a 40% less luciferase activity than WT. *LDLR*, *c.*19G>A*, *c.*503C>T*, *c.*517C>A* and *c.*1227C>T* did not show significant differences in luciferase activity with respect to wild type (WT).

Conclusions: In this study, we found that *c.*653G>C* variant reduce the expression of LDLR being probably the cause of the hypercholesterolemia in our patient. The 3'UTR region of *LDLR* gene should be explored in order to find variants with the potential for reducing the expression of LDLR. Further studies should be performed to clarify the involved mechanism.

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P06.025.B The rs113883650 variant of *SLC7A5 (LAT1)* gene may alter brain vulnerability to hyperphenylalaninemia

Miroslaw Bik-Multanowski, *Kinga Bik-Multanowska, Iwona Betka, Anna Madetko-Talowska*

Jagiellonian University, Krakow, Poland.

Introduction: In the individuals diagnosed with phenylketonuria (PKU) brain damage can be caused by the absence of effective dietary treatment. Nevertheless, several reports exist describing interindividual differences of the brain vulnerability to the toxic influence of hyperphenylalaninemia. This might result from alteration of the kinetics of phenylalanine across the blood-brain barrier, which is regulated by the LAT1 transporter.

Patients and Methods: We assessed the effect of carriership of the common variant rs113883650 of the SLC7A5 (LAT1) gene on brain phenylalanine content. We used magnetic resonance spectroscopy to measure the intensity of the brain phenylalanine signal in a group of 30 PKU patients aged 12-25 years. Next, we compared the results obtained in carriers of the rs113883650 variant with the wild-type individuals.

Results: Genotyping of the SLC7A5 (LAT1) gene identified 17 wild type individuals, 12 heterozygotes and one homozygote with

regard to the rs113883650 variant. On the day of magnetic resonance spectroscopy examination, all the patients revealed very high blood phenylalanine concentration that is typical for untreated PKU. The mean intensity of the brain phenylalanine signal was significantly higher in the carriers of the rs113883650 variant compared to the wild-type individuals ($p = 0.0022$).

Conclusions: Our findings show that carriership of the rs113883650 variant of the SLC7A5 gene has a potential to increase the concentration of brain phenylalanine in PKU patients with severe hyperphenylalaninemia. This could to some extent explain the unusually mild clinical course in some untreated patients. The study was sponsored by the National Science Centre, Poland (project number 2018/29/B/NZ5/01215).

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P06.026.C ZOEMBA: combining metabolomics and genomics data to solve the unsolved

Machteld M. Oud[1], *Saskia N. van der Crabben*[2], *Elise A. Ferreira*[2], *Marielle Alders*[2], *Judith J. Jans*[3], *Nanda M. Verhoeven*[3], *Karlien L. M. Coene*[1], *Leo A. J. Kluijtmans*[1], *Udo F. H. Engelke*[1], *Eduard Struys*[2], *Lisenka E. L. M. Vissers*[1], *Saskia B. Wortmann*[1], *Hans R. Waterham*[2], *Mirjam Langeveld*[2], *Clara D. M. van Karnebeek*[1]

[1]*Radboudumc, Nijmegen, Netherlands,* [2]*Amsterdam UMC, Amsterdam, Netherlands,* [3]*University Medical Center Utrecht, Utrecht, Netherlands.*

Introduction: Inherited metabolic disorders (IMD) are rare disorders caused by defects in biochemical processes. Early diagnosis of IMDs is key as many are amenable to treatment. Despite diagnostic advances, ~50% of patients remain undiagnosed. ZOEMBA is a Dutch multicenter study of our UMD consortium that aims to establish a diagnosis in 500 IMD patients via integrated multi-omics analysis.

Materials and Methods: Patient inclusion criteria are: clinical or biochemical suspicion of an IMD and no diagnosis after extensive genetic and metabolic work-up. Deep phenotyping, WES re-analysis, WGS analysis and untargeted metabolomics will be performed. Leads resulting from metabolomics are checked in the genomics dataset and vice versa to pinpoint the underlying pathophysiology.

Results: In the first year, we enrolled 21 patients with a carefully characterized IMD phenotype for whom WES re-analysis and untargeted metabolomics was performed. Re-analysis of WES data has yielded a diagnosis in two patients. In a girl with neuroregression, WES re-analysis revealed two bi-allelic variants in a recently identified disease gene *TMPRSS9*. Secondly, WES re-analysis for an adult male has revealed two homozygous variants in *ApoE* that most likely explain the patient's phenotype.

Conclusions: We were able to show the benefit of deep phenotyping and WES re-analysis with a yield of 10%. We aim to enroll 500 patients and complete the dataset with WGS and metabolomics data to explore the added value of these technologies. Altogether, this will enable us to discover novel genes, phenotypes and evaluate the usefulness of integrated multi-omics data in clinical practice.

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P06.027.D Novel missense variant in the *INSR* gene in Russian patient with metabolic condition: correction of the diagnosis

Natalya V. Tarasenko[1,2], *Nadezda P. Babushkina*[1], *Tatiyana V. Saprina*[2], *Tatiyana A. Milovanova*[2], *Kseniya V. Trubchenko*[2], *Maria V. Golubenko*[1], *Maria S. Nazarenko*[1]

[1]*Research Institute of Medical Genetics, Tomsk National Research Medical Center of the Russian Academy of Sciences, Tomsk, Russian Federation,* [2]*Siberian State Medical University, Tomsk, Russian Federation.*

Introduction: We have performed clinical exome sequencing in a patient with preliminary diagnosis of maturity onset diabetes of the young subtype 2 (MODY2, OMIM 125851). Onset of the disease occurred at the age of 36 with episodes of hyper- and hypo-glycaemia.

Materials and Methods: DNA was extracted from the blood leucocytes of the patient. Massive parallel sequencing was performed on NextSeq550 (Illumina) with Clinical Exome Solution™ exome panel (SOPHiA GENETICS). Data analysis and variant annotations were done with SOPHiA AI™ and SOPHiA DDM™ (SOPHiA GENETICS).

Results: No pathogenic mutations were revealed in the genes which are casual for MODY subtypes (OMIM 606391 - *HNF4A*, *HNF1A*, *GCK*, *PDX1*, *HNF1B*, *NEUROD1*, *KLF11*, *CEL*, *PAX4*, *INS*, *BLK*, *ABCC8*, *KCNJ11*, *APPL1*, *PCBD1*, *TRMT10A*). However, there were several variants in other genes that require further investigation. In particular, heterozygous nonsynonymous substitution in the *INSR* gene (GRCh37/hg19, chr19 (19p13.2):7174702, c.DNA: c.1015T>C, protein: p.Cys339Arg) was identified, and it was confirmed by Sanger sequencing. This substitution is absent in ExAc database and has been classified as variant with uncertain significance. To confirm absence of the variant in the populations, we have genotyped population samples of European origin ($N = 327$) and of Asian origin (Tuvinians and Yakuts, $N = 224$) consisting of peoples living in Siberia. There were no occurrence of this substitution in the samples.

Conclusion: It is known that *INSR* mutations may cause several genetic syndromes. Heterozygous status for the Cys339Arg variant together with clinical symptoms suggests that the patient has autosomal dominant familial hyperinsulinemic hypoglycemia type 5 (OMIM 609968).

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P06.028.A Pancreatic expression of genes connected to glucose metabolism - nutrigenetic regulation

Ivelina Mihaleva, *Margarita Strokova, Eliana Dimova, Pavlina Gateva, Ivanka Dimova*

Medical University of Sofia, Sofia, Bulgaria.

Background: Ketogenic diet (KD) is low-carbohydrate, high-fat diet used for different health-related effects. It has positive effects on cardiovascular parameters, affects body adiposity and improves features of metabolic syndrome in humans. Although studies evaluating the efficacy and metabolic effects of KD have increased recently, the effects of macronutrient-controlled diets remain controversial. The objective of our study was to analyze the expression levels of genes related to glucose metabolism/insulin action, in pancreas of mice fed with KD with and without Vitamin D for 1 month compared to mice on normal diet.

SPRINGER NATURE

Materials and methods: Separation of two groups of mice of at least n = 10 on a standard diet and on a KD +/- Vitamin D. After 1 month, RNA was isolated from pancreas and after reverse transcription, the following genes were analyzed by real-time PCR: *INS*, *GCK*, *ABCC8* and *KCNJ11*.

Results: We established a significant decrease in the pancreatic expression levels for all genes, especially Insulin gene, after KD.

Gene	Relative pancreatic expression (RQ)
Ins1	0.1
ABCC8	0.21
KCNJ11	0.32
Gck	0.54

After KD with Vitamin D, we revealed an increase in pancreatic expression of Insulin gene compared to group of KD only (RQ = 0.35), not reaching the level in controls.

Conclusion: KD significantly reduces the insulin expression in the pancreas, which could be increased by administration of Vitamin D.

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P06.030.C The Chylomicronemia Syndrome: a case of familial partial lipodystrophy type 3 associated with a pathogenic variant in *PPARG*

*Sonia Rodriguez-Novoa[1], Carmen Rodríguez Jiménez[1], Javier Sanguino[1], A. Viejo-LLorente[2], A.E. Kerguelén-Fuentes[2], D. Hernández-Maraver[2], A. Barros-Campos[3], **P. Martínez-Hernández**[4]*

[1]Metabolic Disease Laboratory, Genetic Department. Hospital Universitario La Paz, Madrid, Spain. Group of dislipemias of genetic origin and metabolic diseases, IdiPAZ, Hospital Universitario La Paz, Madrid, Spain., Madrid, Spain, [2]Apheresis Unit, Hematology Department, Hospital Universitario La Paz, Madrid, Spain, Madrid, Spain, [3]Institute of Medical and Molecular Genetics, IdiPAZ, Hospital Universitario La Paz /& CIBERER, Unit 753, ISCIII, Madrid, Spain., Madrid, Spain, [4]Internal Medicine, Hospital Universitario La Paz, Madrid, Spain., Madrid, Spain.

Introduction: The Chylomicronemia Syndrome (QS) is characterized by severe hypertriglyceridemia (>1000 mg/dL or >11.3 mmol/L), abdominal pain, recurrent acute pancreatitis, eruptive xanthomas, and lipemia retinalis. Its causes are variable including secondary forms of hypertriglyceridemia, (multifactorial chylomicronemia syndrome-MFCS); LPL deficiency (familial chylomicronemia syndrome, FCS), or familial partial lipodystrophy (FPLD). We present the case of a patient with QS associated with FPLD.
Patient and methods: A 22-years-old African American female, was referred due to severe hypertriglyceridemia and eleven episodes of acute pancreatitis (the first one at age 15 yrs), with highest and lowest triglycerides levels of 6009 mg/dl (67.9 mmol/L) and 129 mg/dL (1.46 mmol/L), respectively. She had generalized *acanthosis nigricans* (neck, axillae, elbows, groins and knees), BMI 21.38kg/m2, and reduced fat in the face, upper and lower extremities. She was treated with low-fat diet, insulin, fibrates, omega-3 fatty-acids and several sessions of apheresis during pancreatitis episodes. Adherence to diet was irregular, she drank beer intermittently, and during the last year she also used oral contraceptives for six months. Targeted NGS analysis was performed with a customized panel including 500 genes related with metabolic diseases.

Results: A heterozygous missense pathogenic variant in *PPARG*, NM_015869.4:c.452A>G, p.(Tyr151Cys), previously associated with FPLD type 3, was identified.

Conclusion: Patients with *PPARG* mutations may develop lipodystrophy due to defective adipocyte differentiation. In previous studies, the p.(Tyr151Cys) *PPARG* variant showed impaired DNA-binding capacity and hence reduced transcriptional activity. Although FPLD is a rare condition, it should be taken into consideration in patients with severe hypertriglyceridemia and recurrent pancreatitis.

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P06.031.D The utility of reverse phenotyping: A case of lysinuric protein intolerance presented with childhood osteoporosis

*Enise Avci Durmusalioglu[1], Esra Isik[1], Durdugul Ayyildiz Emecen[1], Damla Goksen Simsek[2], Samim Ozen[2], Huseyin Onay[3], Melis Kose[1], Tahir Atik[1], Sukran Darcan[2], Ozgur Cogulu[1], **Ferda Ozkinay**[1]*

[1]Pediatric Genetics Subdivision, Department of Pediatrics, Faculty of Medicine, Ege University, Izmir, Turkey, [2]Department of Pediatric Endocrinology and Diabetes, Faculty of Medicine, Ege University, Izmir, Turkey, [3]3Department of Medical Genetics, Faculty of Medicine, Ege University, Izmir, Turkey.

Introduction: Childhood osteoporosis is often a consequence of a chronic disease or its treatment. Lysinuric protein intolerance (LPI), a rare secondary cause of the osteoporosis, is an autosomal recessive disorder with clinical features ranging from nearly normal growth with minimal protein intolerance to severe multisystemic involvement. This disorder is caused by biallelic mutations in the *SLC7A7* gene. Due to the clinical variability of the disease, diagnosis can be difficult; with misdiagnosis also a possibility. We report a case diagnosed to have LPI using a Next Generation Sequencing (NGS) panel and evaluate the utility of reverse phenotyping.

Case Report: A fifteen-year-old-boy with an initial diagnosis of osteogenesis imperfecta, was referred to our clinic due to a number of atypical findings accompanying to osteoprosis such as splenomegaly and bicytopenia, He was the first child of nonconsanguineous parents; however, his parents originated from the same village. On physical examination, he had asthenic body build and splenomegaly. His laboratory tests showed leukopenia, thrombocytopenia, elevated serum lactate dehydrogenase and ferritin levels. Bone marrow aspiration revealed a hemophagocytosis. A NGS panel (TruSight One Sequencing Panel) was performed and a novel homozygous mutation of c.257G>A (p.Gly86Glu) in *SLC7A7* gene (NM_001126106.2) was detected. A reevaluation of patient's past medical history and phenotypic features revealed that he avoided eating protein-rich food while experiencing recurrent diarrhea attacks during infancy. Laboratory tests showed excess urinary excretion of cationic amino acids supporting lysinuric protein intolerance.

Conclusion: Reverse phenotyping using a targeted gene panel shortens the diagnostic process in patients with mild phenotypes.

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P06.032.A Extended phenotype studies in carriers and potentially affected individuals of selected lysosomal storage diseases among the participants of the Estonian Biobank

Ellen Rebecca Kruuse[1], *Marili Palover*[1], *Margit Nõukas*[1], *Tiit Nikopensius*[1], *Neeme Tõnisson*[1,2]

[1]*Estonian Biobank, Institute of Genomics, University of Tartu, Tartu, Estonia,* [2]*Department of Clinical Genetics, United Laboratories, Tartu University Hospital, Tartu, Estonia.*

Background: Lysosomal storage diseases (LSDs) are individually rare, but collectively constitute a considerable part of hereditary metabolic diseases. Due to low prevalence and phenotypic variability of individual LSDs, they may be underdiagnosed or misdiagnosed, especially in atypic late onset cases.

We have selected a subset of LSDs based on the availability of specific enzyme replacement therapy: Pompe disease (*GAA* gene), Fabry disease (*GLA* gene), Gaucher disease (*GBA* gene) and Mucopolysaccharidosis Type I (*IDUA* gene).

The aim of this study is to: i) identify individuals with a possible genetic risk for LSDs, ii) assess the phenotype by enzyme analysis in biobank participants and iii) to disclose relevant findings to individuals at genetic risk for LSDs.

Results: By using 'genotype-first' approach, we screened the cohort of Estonian Biobank (n = 150K) for pathogenic or potentially pathogenic variants in the respective genes. We identified 11 individuals with potentially clinically relevant findings: 1 compound heterozygote and 5 alternative homozygotes for the *GAA*, 1 alternative homozygote for the *GBA* and 4 hemizygotes for the *GLA* gene.

Conclusions: We have set up a framework for re-contacting biobank participants, in order to determine the phenotype by clinical diagnoses and enzyme activity analyses and return of genetic information upon consent. LSDs are a heterogenous group of diseases, that would benefit from further studies using the biobank data.

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P06.035.D Modern approaches to the diagnosis of Methylmalonic academia/aciduria

Vita Antsupova[1], *Iryna Lastivka*[2], *Larysa Sheiko*[3], *Ljudmila Brisevac*[3], *Iana Ushko*[1], *Volodymyr Davydiuk*[4]

[1]*Bohomolets National medical university, Kyiv, Ukraine,* [2]*Bukovinian State Medical University, Chernivtsi, Ukraine,* [3]*Shupyk National Medical Academy of Postgraduate Education, Kyiv, Ukraine,* [4]*National Pirogov Memorial Medical University, Vinnitsa, Ukraine.*

Introduction: Methylmalonic acidemia/aciduria (MMA) is a genetically heterogeneous inherited disease from the group of organic acidemias. Clinically manifests metabolic ketoacidosis, mental and physical retardation. Differential molecular diagnosis of MMA is important for the choice of treatment tactics.

Materials and Methods: A clinical case of methylmalonic acidemia. Routine and modern biochemical diagnostic methods (tandem mass spectrometry (TMS), gas chromatography (GC), high performance liquid chromatography (HPLC)) were used to establish the diagnosis; molecular genetic research.

Results: An 18-month-old girl was referred to a geneticist. Preliminary diagnosis "Metabolic disorders of unclear genesis, ketoacidotic syndrome, neuroarthritic constitution, anemia of the first degree." The girl underwent a comprehensive examination, which included determination of the profile of blood acylcarnitines by TMS, blood amino acids by HPLC; determination of renal excretion of organic acids by GC; determination of the level of lactate, blood ammonia, glycemic profile; molecular genetic research. An increase in the concentration of propionylcarnitine, glycine, cystine and Proline was detected in the blood; the presence of methylmalonic acid in the urine. A molecular genetic study was performed, a mutation in c.655A>T (p.Asn219Tyr) of the MUT gene was detected. Final diagnosis: Methylmalonic acidemia vitamin B12-intact form.

Conclusions: Diagnosis of MMA presents certain difficulties due to the clinical heterogeneity of symptoms and nonspecific manifestation of the disease. For the diagnosis of MMA, the most informative are the data of the profile of blood acylcarnitines and the determination of the presence of MMC in the urine. The final diagnosis is based on the results of molecular genetic studies.

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P06.036.A A novel homozygous missense mutation in UQCRC2associated with severe encephalomyopathy, mitochondrial complex III assembly defect and activation of mitochondrial protein quality control

Daniela Burska[1], *Lukas Stiburek*[1], *Jana Krizova*[1], *Marie Vanisova*[1], *Vaclav Martinek*[2], *Tomas Honzik*[1], *Jiri Zeman*[1], *Hana Hansikova*[1], ***Marketa Tesarova***[1]

[1]*Department of Pediatrics and Adolescent Medicine, First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague 2, Czech Republic,* [2]*2Department of Biochemistry, Faculty of Science, Charles University, Prague 2, Czech Republic.*

The mitochondrial respiratory chain (MRC) complex III (CIII) associates with complexes I and IV (CI and CIV) into supercomplexes. We identified a novel homozygous missense mutation (c.665G>C; p.Gly222Ala) in *UQCRC2* coding for structural subunit Core 2 in a patient with severe encephalomyopathy. The structural data suggest that the Gly222Ala exchange might result in an altered spatial arrangement in part of the UQCRC2 subunit, which could impact specific protein-protein interactions. Accordingly, we have found decreased levels of CIII and accumulation of CIII-specific subassemblies devoid of UQCRC1, UQCRC2, and UQCRFS1 subunits in the patient's fibroblasts. The lack of UQCRC1 subunit-containing subassemblies could result from an impaired interaction with mutant $UQCRC2^{Gly222Ala}$ and subsequent degradation of both subunits by mitochondrial proteases. Observed elevated amount of matrix CLPP protease suggests the activation of the mitochondrial protein quality control machinery in UQCRC2-Gly222Ala fibroblasts. Data revealed a rate-limiting character of CIII availability for the supercomplex formation, accompanied by a diminished amount of CI. Furthermore, we found impaired electron flux between CI and CIII in skeletal muscle and fibroblasts of the patient. The ectopic expression of wild-type UQCRC2 in patient cells rescued maximal respiration rate, demonstrating the deleterious effect of the mutation on MRC. Our study expands the phenotypic spectrum of CIII Core protein deficiency, provides

insight into the assembly pathway of human CIII, and supports the requirement of assembled CIII for a proper accumulation of CI. *This work was supported by the Ministry of Health of the Czech Republic (grants AZV 17-30965A, NV19-07-00149, RVO VFN 64165).*

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P06.037.B Prevalence and clinical prediction of mitochondrial disorders in a large neuropediatric cohort

Amelie T. van der Ven[1], *Jessika Johannsen*[1], *Matias Wagner*[2], *Konstantinos Tsiakas*[1], *Tatjana Bierhals*[1], *Fanny Kortüm*[1], *Davor Lessel*[1], *Theresia Herget*[1], *Katja Kloth*[1], *Jasmin Lisfeld*[1], *Tasja Scholz*[1], *Nadia Obi*[1], *Saskia Wortmann*[2], *Holger Prokisch*[2], *Christian Kubisch*[1], *Jonas Denecke*[1], *René Santer*[1], *Maja Hempel*[1]

[1]*UKE Hamburg-Eppendorf, Hamburg, Germany,* [2]*TUM Munich, Munich, Germany.*

Introduction: Mitochondriopathies constitute a clinically important subgroup of (neuro-)pediatric disorders. Early identification of mitochondriopathies is desirable due to a potentially rapid clinical decline and the availability of treatment options in selected conditions. Identified patients should preferentially be selected for expedited genetic diagnostics yielding molecular diagnosis within a few days in comparison to several weeks when conducted in a routine clinical setting. We here determined the prevalence of molecularly confirmed mitochondriopathies in a large cohort of undiagnosed neuropediatric patients and compared an established clinical rating tool (MDC) as well as a newly composed, simplified version of the existing tool (MDC-NP) in regard to their predictive capabilities.

Methods: 491 unrelated children with neurological symptoms underwent a comprehensive diagnostic work-up including exome sequencing. Identified disease-genes were dichotomized depending on relevance to mitochondrial function. Rating tools were applied using standardized phenotype information collected for each patient.

Results: The molecular solve rate within our cohort was 51%. In 12% of solved cases, a mitochondriopathy-associated gene was found to harbor the disease-causing variant. The MDC score predicted the underlying mitochondriopathy-associated genotype with a sensitivity of 0.59 (0.41-0.75) and a specificity of 0.99 (0.96-1.00). The newly composed MDC-NP-tool, in contrast, exhibited a significantly higher sensitivity (0.83; 0.65-0.93) and a specificity of 0.96 (0.92-0.98).

Conclusion: Mitochondriopathies constitute a numerically significant subgroup of neuropediatric patients. We introduce the MDC-NP score as simplified and sensitive bedside screening tool for rapid identification of children with mitochondriopathies. Exome Sequencing was partially funded by BMBF and GENOMIT (mitoNET 01GM1113C and 01GM1207 to H.P.).

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P06.038.C A novel mutation of *PDP1* gene in a pediatric patient

Flavia Anne-Elise Szekely[1], *Adela Chirita-Emandi*[2], *Cristian Zimbru*[3], *Nicoleta Andreescu*[2], *Maria Puiu*[2]

[1]*Emergency Clinical County Hospital "Pius Branzeu", Timisoara, Romania,* [2]*Center of Genomic Medicine, University of Medicine and Pharmacy "Victor Babes", Regional Center of Medical Genetics Timis, Clinical Emergency Hospital for Children "Louis Turcanu", part of ERN ITHACA, Timisoara, Romania,* [3]*Center of Genomic Medicine, University of Medicine and Pharmacy "Victor Babes", 4. Department of Automation and Applied Informatics Politehnica University, Timisoara, Romania.*

Introduction: Mitochondrial diseases are a group of heterogeneous inherited metabolic diseases, caused by a dysfunction of the mitochondrial respiratory chain. Mitochondria is an intracellular organelle, which produce the energy molecule storage adenosine triphosphate. We aim to present a novel pathogenic variant in *PDP1* gene in a pediatric patient.

Material and methods: A 2-year-old female patient presented two episodes of coma due to metabolic acidosis associated with high level of lactic acid, hyperglycemia, hypothermia, without inflammatory syndrome. Acylcarnitine profiles were altered in the patient. Biological sample (blood) was collected and Next Generation Sequencing was performed, using Illumina TruSight One sequencing panel, which includes 4813 genes.

Results: Next generation sequencing analysis revealed a homozygous missense variant NM_001161780.1 (PDP1):c.1288C>T. The stopgain variant is previously unreported, has very low frequency in population and creates a premature stop codon NP_001155252.1:p.(Arg430Ter), thus has been classified as pathogenic.

Conclusion: PDP1 gene encodes pyruvate dehydrogenase phosphatase (PDP1), which is one of the two PDP isoforms. The role of this gene is to regulate the activity of the mitochondrial multienzyme pyruvate dehydrogenases complex (PDC). Pyruvate dehydrogenase phosphatase deficiency (PDHPD), caused by homozygous mutation in the PDP1 gene, is characterized by neonatal/infantile and childhood lactic acidosis, elevated plasma alanine, delayed psychomotor development, epileptic encephalopathy and hypotonia. There are few case reports suggesting the importance of this enzyme complex malfunction as triggers for lactic acidemia with elevated serum lactate levels. The homozygous stopgain variant identified in *PDP1* gene, is a novel cause associated with pyruvate dehydrogenase complex deficiency.

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P06.039.D Novel FARS2 variants in patients with early onset encephalopathy with or without epilepsy associated with long survival

Giulia Barcia, Marlene Rio, Zahra Assouline, Coralie Zangarelli, Charles-Joris Roux, Pascale de Lonlay, Julie Steffann, Isabelle Desguerre, Arnold Munnich, Jean-Paul Bonnefont, Nathalie Boddaert, Agnès Rötig, Metodi D. Metodiev, ***Benedetta Ruzzenente***

Institute Imagine, Paris, France.

Mitochondrial translation is essential for the biogenesis of the mitochondrial oxidative phosphorylation system (OXPHOS) that synthesizes the bulk of ATP for the cell. Hypomorphic and loss-of-function variants in either mitochondrial DNA or in nuclear genes that encode mitochondrial translation factors can result in impaired OXPHOS biogenesis and mitochondrial diseases with variable clinical presentations. Compound heterozygous or homozygous missense and frameshift variants in the FARS2 gene, that encodes the mitochondrial phenylalanyl-tRNA synthetase, are commonly linked to either early-onset epileptic mitochondrial encephalopathy or spastic paraplegia. Here, we expand the genetic spectrum of FARS2-linked disease with three patients

carrying novel compound heterozygous variants in the FARS2 gene and presenting with spastic tetraparesis, axial hypotonia and myoclonic epilepsy in two cases.

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P06.040.A Mitochondrial bioenergetics and cardiolipin re-modeling deregulation in mitochondrial trifunctional protein (TFP) deficiency

Eduardo Vieira Neto[1,2], *Meicheng Wang*[1,2], *Yudong Wang*[1,2], *Tamil S. Anthonymuthu*[1,3,4,5], *Hülya Bayir*[1,3,4,5], *Jerry Vockley*[1,6]

[1]*University of Pittsburgh, Pittsburgh, PA, USA,* [2]*UPMC Children's Hospital of Pittsburgh, Pittsburgh, PA, USA,* [3]*Children's Neuroscience Institute, UPMC Children's Hospital of Pittsburgh, Pittsburgh, PA, USA,* [4]*Safar Center for Resuscitation Research, University of Pittsburgh, Pittsburgh, PA, USA,* [5]*Center for Free Radical and Antioxidant Health, University of Pittsburgh, Pittsburgh, PA, USA,* [6]*Center for Rare Disease Therapy, UPMC Children's Hospital of Pittsburgh, Pittsburgh, PA, USA.*

Introduction: Mitochondrial trifunctional protein (TFP) catalyzes three steps in fatty acid β-oxidation. Mutations in α (*HADHA*) or β (*HADHB*) subunits can result in general TFP deficiency, while the *HADHA* p.Glu510Gln mutation accounts for isolated 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency. The α subunit has also monolysocardiolipin acyltransferase activity required for cardiolipin (CL) re-modeling. This study aimed to characterize mitochondrial bioenergetics and CL content in fibroblasts from TFP/LCHAD deficiency patients.

Materials and Methods: Mitochondrial bioenergetics were assessed by oxygen consumption rate with a Seahorse XFe96 Extracellular Flux Analyzer. Mitochondria phospholipids (including CL) were measured by LC-MS/MS. Acylcarnitine profiles were determined by ESI-MS/MS.

Results: All patient fibroblasts had lower rates of maximal respiration, and spare respiratory capacity, while basal and ATP-linked respiration rates were variable, compared to controls. Levels of mature CLs in mitochondria were decreased, while those of monolyso-CLs were increased. The changes in CLs and monolyso-CLs differed among cells. All mutant cells had higher levels than control of the acylcarnitine marker C16-OH.

Conclusions: There was a clear reduction of mitochondrial respiration in patient fibroblasts, although some were able to meet their baseline energy demand. Isolated LCHAD deficiency fibroblasts had an abnormal CL profile as did two other cells - one with a splicing mutation in *HADHA*, and the other with a small deletion in *HADHB* leading to shift in reading frame. This study indicates that there is a correlation between CL profiles and mitochondrial bioenergetics in cells with *HADHA* and *HADHB* mutations, a finding that offers new potential therapeutic options for patients.

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P06.041.B Re-evaluation of gene pathogenicity for MODY

Thomas W. Laver[1], *Matthew N. Wakeling*[1], *Olivia Knox*[1], *Kevin Colclough*[2], *Caroline Wright*[1], *Sian Ellard*[1], *Andrew T. Hattersley*[1], *Michael N. Weedon*[1], *Kashyap A. Patel*[1]

[1]*University of Exeter Medical School, Exeter, United Kingdom,* [2]*Royal Devon & Exeter NHS foundation trust, Exeter, United Kingdom.*

Introduction: Sequencing data for large control populations and disease cohorts are needed to accurately assess the pathogenicity of genes causing rare monogenic disorders. Concern has been raised for *BLK*, *KLF11* and *PAX4* as causes of MODY - a dominant form of monogenic diabetes. In this study we re-evaluated whether *BLK*, *KLF11* and *PAX4* cause MODY.

Materials and methods: We examined case level genetic evidence (variant frequency in population and co-segregation with diabetes) for all published variants in *BLK*, *KLF11* and *PAX4*. We performed a gene burden test for ultra-rare non-synonymous variants (MAC = 1) in 1227 probands with MODY and 185,898 control individuals from the population-based UKBiobank study. We also assessed *HNF1A* and *HNF4A* variants (well-established causes of MODY) and conducted multiple sensitivity analysis (different control cohorts, synonymous variant enrichment) to validate our results.

Results: The published variants showed poor co-segregation with diabetes (combined LOD scores: *BLK* 1.16, *KLF11* 1.2, *PAX4* <1.2) in contrast to well established genes (*HNF1A* 9.63, *HNF4A* 15.05). The early published variants in these genes are common in gnomAD v2.1.1: 9/11 variants above the disease prevalence (1.08 in 10,000). Ultra-rare non-synonymous variants were not enriched in a MODY cohort compared to the UKbiobank cohort (PTVs $P>0.05$, missense $P>0.1$ for all three genes) in contrast to well established genes ($P < 2.79E-06$ for *HNF1A* and *HNF4A*). Sensitivity analyses using different control cohorts and synonymous variants supported our results.

Conclusions: Rare variants in *BLK*, *KLF11* and *PAX4* do not cause MODY and should not be included in diagnostic testing for MODY.

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P06.042.C Mitochondrial Membrane Protein - Associated Neurodegeneration: a rare variant and a difficult diagnosis

Alexandru Caramizaru[1], *Mihai Cucu*[2], *Cristina Durac*[1], *Raluca Tutunaru*[1], *Andrada Gheorghe*[1], *Bianca Petre-Mandache*[1], *Amelia Dobrescu*[2]

[1]*CRGM Dolj, Craiova, Romania,* [2]*CRGM Dolj, UMF Craiova, Craiova, Romania.*

Introduction: Mitochondrial Membrane Protein-Associated Neurodegeneration (MPAN) is one of the major forms of NBIA, with an estimated worldwide prevalence of about 1/1,000,000 (less than 80 cases reported to date). MPAN is caused by mutations in the C19orf12 gene (19q13.11). A founder mutation (c.204_214del11; p. Gly69ArgfsX10) has been described in Eastern Europe.

Materials and Methods: We aim to present the case of a 22 year old male patient who initially presented with walking difficulties, trauma caused by falling and bilateral optic atrophy. The onset was around 13-14 years of age. Progressively, the symptomatology also included equilibrium problems, moderate spasticity, distal amiotrofic motor deficit of the upper and lower limbs, bilateral amiotrophy of the quadriceps muscle, bilateral pes cavus, mild scoliosis, postural tremor and writing difficulties. EMG showed a motor axonal neuropathy and MRI revealed a hypodense aspect of the grey nuclei. Furthermore, molecular analyses were performed.

Results: An NGS panel for CMT genes and HSPB1, BSCL2, GARS and REEP1 was negative. A homozygous variant (nonsense

substitution) in the C19orf12 gene, (p.Leu72) Chr19(GRCh37): g.30193863A>C, was identified through WES.

Conclusions: When approaching a neurodegenerative pathology characterized by a non-specific combination of signs and symptoms, the association of particularities such as optical atrophy and hypodensity of grey nuclei could be a key for the diagnosis, and could further prevent long and expensive genetic tests. The identification of an unreported C19orf12 homozygous mutation (c.215T>G) in a patient without any family history of consanguinity suggests the possibility of a novel variant characteristic to the population in this region.

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P06.043.D Mitochondrial DNA mutations do not impact early human embryonic development

Kalliopi Chatzovoulou[1], *Anne Mayeur*[2], *Nadine Gigarel*[1], *Fabienne Jabot-Hanin*[1], *Laetitia Hesters*[2], *Arnold Munnich*[1], *Nelly Frydman*[2], *Jean-Paul Bonnefont*[1], *Julie Steffann*[1]

[1]*Imagine Institute, Paris, France,* [2]*Antoine Beclere Hospital, Clamart, France.*

Introduction: mtDNA mutations are a frequent cause of devastating metabolic disorders. Their presence might induce fertilization defects or embryo development failure in oocytes or preimplantation embryos, respectively. Whether a metabolic rescue through an increase of mtDNA amount in mutant embryos takes place during cleavage-stage human embryonic development remained unclear.

Materials and Methods: Preimplantation genetic testing was performed for 55 couples. In total, 165 embryos at risk of carrying a non-metabolic, non-mitochondrial genetic disorder (control group), and 16 embryos at risk of carrying a mtDNA mutation (mitochondrial group), were included. mtDNA amount was quantified on DNA from blastomeres by real-time PCR. Embryonic quality was evaluated at day-3 and day-4/5. Embryonic viability was defined as the ability of an embryo to implant and give a viable pregnancy.

Results: Maternal age and antral follicle count were similar between control and mitochondrial groups, except from anti-Müllerian hormone levels. Maternal age was not correlated to the blastomere mtDNA copy number. No significant difference in either quality or viability between control and mitochondrial embryos was found. A significant variability of the mtDNA amount between sister blastomeres was observed in both embryonic groups. No modification of the blastomere mtDNA amount was found when the mother carried a mtDNA mutation. Finally, mtDNA copy number was not correlated to mutant loads.

Conclusions: A pathogenic mtDNA mutation does not modify the mtDNA metabolism in human cleavage-stage embryos, suggesting the absence of negative selection at this stage. Funding: This work was supported by the "Association Française contre les Myopathies".

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P06.045.B A case of Mucopolysaccharidosis type 2 in a girl

Nataliia Samonenko[1,2], *Nataliia Trofimova*[1], *Nataliia Mytcyk*[1], *Svetlana Kormoz*[1], *Irina Gregul*[1], *Nataliia Olhovich*[1], *Nataliia Pichkur*[1], *Olena Okhotnikova*[2], *Nataliia Gorovenko*[3]

[1]*National Specialise Children Hospital, Kyiv, Ukraine,* [2]*P.L. Shupyk National Medical Academy of Postgraduate Education, Kyiv, Ukraine,* [3]*Institute of Genetic and Regenerative Medicine, Kyiv, Ukraine.*

Background: Mucopolysaccharidosis type II (MPS II) is one of a group of hereditary metabolic diseases. Hunter syndrome a genetically associated to the deficiency of the iduronate 2-sulfatase enzyme (IDS). It is an X-linked recessive disorder and occurs predominantly in males. IDS is responsible for the breakdown of glycosaminoglycans. Decreased activity of IDS results accumulation of heparan sulfate and dermatan sulfate in multiple organs of the body.Manifestations of the MPS II in girls are extremely rare and are associated with the inactivation of one chromosome (Lyonization effect).Case Report: Herein we present an 3,5 years old girl admitted with mental and speech developmental disorders, behavioral disorders, changes in appearance, contractures of the joints, umbilical hernia, scoliosis, short stature, hepatosplenomegaly. Examination revealed bilateral secretory otitis, conductive deafness, tunnel syndrome. This girl is the first in the family. She has a younger healthy brother. From 1 month old it was observed by doctors with macrocephaly, umbilical hernia. At the age of 8 m, stiffness of joints was already observed. Developmental delay was at 1,5 years old. The examination revealed a significant increase in the excretion of glycosaminoglycans with dermatan sulfate in urine. Iduronate sulfatase activity is absent.Molecular genetic work-up heterozygous c.797_798del gene IDS (p.(Pro266Leufs*75). No gene mutations were detected in the mother or father.

Discussion: Mucopolysaccharidosis type 2 can occur in girls. In the presence of specific symptoms should not forget about additional diagnosis and exclusion of this type of MPS. The girl was diagnosed with a new mutation for the family.

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P06.046.C Revisiting clinical presentation of Egyptian patients with mucopolysaccharidoses

Solaf Elsayed[1], *Rabah Shawky*[1], *Walaa Yousef*[2], *Abdullah Abdullah*[1]

[1]*Medical Genetics Department, Faculty of medicine, Ain Shams University, Cairo, Egypt,* [2]*Pediatrics Department, Faculty of medicine, Ain Shams University, Cairo, Egypt.*

Introduction: Early recognition of red- flag symptoms and signs of potential patients with Mucopolysaccharidoses (MPS) is mandatory for timely diagnosis and management. Cultural differences could have an impact of which symptom trigger the patient to seek medical advice. The aim of the study was to highlight early symptoms and signs in Egyptian patients with MPS and to assess their main clinical features. Patients and **methods:** Data were retrieved from MPS patients' files and recent clinical examination and investigations were also done.

Results: The study included 40 patients (27 male and 13 females) from 32 families. Their age ranged between one and 30 years with median age of 7 years. The most common type found was MPS type III (37.5%). Median age of diagnosis was 3.8 years ± 2.03. Mean diagnostic delay was 1.71 ± 1.53 years (significantly longer in type IV patients). Hepatomegaly was the most common presentation in type I and VI while "rachitic" like bones was the main presentation in type IV. Speech delay and behavioral changes was the presenting symptom in type III patients while behavioral changes and snoring was most common in type II. Previous affected sibling was the trigger to seek medical advice in 30% of patients with type II.

Conclusion: Red-flags symptoms and signs may differ in relation to culture believes and fears. Unlike previous studies, hepatomegaly was a main concern in our patients due to the high prevalence of viral hepatitis while skeletal deformities are not! as childhood rickets is not rare in Egyptian population.

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P06.047.D Molecular basis of Mucopolysaccharidosis IVA (MorquioA syndrome): a review and classification of *GALNS* gene variants and reporting of 68 novel variants

*Alessandra Zanetti[1], Francesca D'Avanzo[1], Moeenaldeen AlSayed[2], Ana Carolina Brusius-Facchin[3], Yin-Hsiu Chien[4], Roberto Giugliani[5], **Emanuela Izzo**[6], David Kasper[7], Hsiang-Yu Lin[8], Shuan-Pei Lin[8], Laura Pollard[9], Akashdeep Singh[6], Rodolfo Tonin[10], Tim Wood[9], Amelia Morrone[10], Rosella Tomanin[1]*

[1]Laboratory of Diagnosis and Therapy of Lysosomal Disorders, Department of Women's and Children's Health, University of Padova; Fondazione Istituto di Ricerca Pediatrica Città della Speranza, Padova, Italy, [2]King Faisal Specialist Hospital and Research Centre; Faculty of Medicine, Alfaisal University, Riyadh, Saudi Arabia, [3]Medical Genetics Service/HCPA, DR BRASIL Research Group/HCPA, and INAGEMP, Porto Alegre, Brazil, [4]Department of Medical Genetics and Pediatrics, National Taiwan University Hospital, Taipei, Taiwan, [5]Department of Genetics/UFRGS, Medical Genetics Service/HCPA, DR BRASIL Research Group/HCPA, and INAGEMP, Porto Alegre, Brazil, [6]BioMarin Pharmaceutical Inc, Novato, CA, USA, [7]ARCHIMED Life Science GmbH, Leberstraße 20, Vienna, Austria, [8]Division of Genetics and Metabolism, Departments of Pediatrics and Medical Research, MacKay Memorial Hospital, Taipei, Taiwan, [9]Biochemical Diagnostic Laboratory, Greenwood Genetic Center, Greenwood, SC, USA, [10]Molecular and Cell Biology Laboratory, Pediatric Neurology Unit and Laboratories, Meyer Children's Hospital; Department of Neurosciences, Psychology, Pharmacology and Child Health, University of Florence, Florence, Italy.

Mucopolysaccharidosis IVA (Morquio syndrome type A, MPS IVA) is a rare autosomal recessive lysosomal storage disorder caused by mutations in the N-acetylgalactosamine-6-sulfatase (*GALNS*) gene. Reduced/absent GALNS enzyme activity causes impaired degradation of chondroitin-6-sulfate and keratan sulfate and their accumulation in tissues. MPS IVA is a clinically heterogeneous disorder, whose presentation varies from a classical rapidly progressing to a nonclassical form. Delayed diagnosis and late introduction of appropriate management are common. The study objective was to collect, analyze, and uniformly summarize all published *GALNS* gene variants, thus updating the previous review (Morrone A. et al. *Hum Mutat*. 2014;35(11):1271-1279). Moreover, previously unpublished genotypes, communicated by 7 reference laboratories worldwide were included in the analysis. Data were analyzed with respect to most common alleles, geographic distribution, level of homozygosity, and genotype-phenotype correlation. All variants were classified according to their pathogenicity as suggested by the ACMG/AMP. Including those previously published, we identified 446 unique variants, among which 68 were novel, in 1190 subjects (including newborn screening positive subjects). Variants distribution included missense (65.0%), nonsense (8.1%), splicing (7.2%), small deletions (del)/insertions(ins) (7.0%), intronic (4.0%), and large del/ins and complex rearrangements (3.8%). Half (50.4%) of the patients were homozygous, 37.1% were compound heterozygous, and 10.7% had only 1 variant detected. *In silico* analyses were performed to evaluate the pathogenicity of novel variants. All variants were submitted to the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) to make them publicly available. Mutation updates are essential for the correct molecular diagnoses, genetic counselling, and disease management for MPS IVA.

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P06.048.B Niacin therapy improves clinical outcomes with the normalization of metabolic abnormalities in a patient with partial NAXD deficiency

Joshua Manor

BCM, Houston, TX, USA.

Introduction: Inflammation, the driving force behind chronic diseases in humans, is both initiated and repressed by enzymes that utilize $NAD(P)^+$/NAD(P)H. The highly conserved 2-enzyme NAD(P)+ repair pathway eliminates toxic NAD(P)HX metabolites that accumulate in inflammatory stress. Deficiency of either NAXD or NAXE depletes the NAD^+ pool and results in fever-triggered fatal encephalopathic crises (MIM 618321 and 617186, respectively).

Case presentation: An adolescent with early-onset progressive weakness was admitted for exacerbation of symptoms and psychosis following fever. At presentation he could not ambulate, required positive pressure ventilation assistance, could not handle oral secretions or extend his neck. Chromosomal microarray

showed a microdeletion of *NAXD* exons 1-2, and exome sequencing revealed a novel *NAXD* SNV predicted to affect both a splice donor site and the mitochondrial pre-sequence tag but leaving the NAXD endoplasmic reticulum and cytosolic isoforms intact.

Results: At time of acute illness, plasma clinical untargeted metabolomics from our patient showed significant alterations in nicotinamide/NAD^+ metabolism (z score negative 4-6), along with a footprint of peroxisomal-mitochondrial axis dysfunction: alterations of TCA cycle metabolites, branched chain amino acids (z score negative 2-3), plasmalogens, phospholipids, and lysophospholipids (z score negative 2-4). Supportive care and niacin supplementation resulted in normal ambulation and muscle strength, no respiratory support, complete normalization of metabolic abnormalities and decrease in creatine kinase from 17,000 to 700 U/L.

Conclusions: Defects in cofactor repair are likely underdiagnosed. Niacin supplementation can reduce inflammatory stress and provide preventative therapy to reduce morbidity and mortality associated with NAD^+ depletion in NAD(P)HX repair pathway deficiency.

J. Manor: None.

P06.049.B Clinical utility of a sponsored gene panel testing program for pediatric epilepsy and CLN2 disease diagnosis: Results from 10,853 tests

Tiffany Yar Pang[1], *Rebecca Truty Truty*[2], *Dianalee A. McKnight*[2], *Britt Johnson*[2], *Ana Morales*[2], *Sara L. Bristow*[2], *Emanuela Izzo*[1], *Guillermo Seratti*[1], *Sookyong Koh*[3], *Elaine C. Wirrell*[4], *John J. Millichap*[5], *Swaroop Aradhya*[2]

[1]*BioMarin Pharmaceutical Inc, Novato, CA, USA,* [2]*Invitae, San Francisco, CA, USA,* [3]*Emory University School of Medicine, Atlanta, GA, USA,* [4]*Mayo Clinic, Rochester, MN, USA,* [5]*Ann and Robert H. Lurie Children's Hospital, Chicago, IL, USA.*

Neuronal ceroid lipofuscinosis type 2 (CLN2 disease) is a rare, inherited, neurodegenerative lysosomal storage disorder caused by deficient TPP1 activity. CLN2 disease often presents with epilepsy between 2 and 4 years of age, accompanied by history of language delay; however, diagnostic delays are common. Behind the Seizure® (BTS) is a US-based, sponsored testing program for children with suspected genetic epilepsy, initiated with the goal to help lower the age of CLN2 disease diagnosis. Individuals were eligible for testing through BTS if they were aged 24-60 months with unprovoked seizure onset at/after 24 months (Dec 2016-Feb 2019) or, following program expansion, aged 0-60 months (Feb 2019-Jan 2020) or 0-108 months (Jan-Nov 2020) with unprovoked seizures onset at any age. Between Dec 2016 and Nov 2020, a total of 10,853 tests were conducted through BTS. The molecular diagnostic yield was 13.7% overall (n = 1485; 102 genes) and 0.18% for *TPP1* (n = 20). In the subset of individuals tested through BTS who were aged 24-60 months with seizure onset at or after 24 months (n = 3,263), the molecular diagnostic yield was 7.9% overall (n = 259) and 0.61% for *TPP1* (n = 20). Mean age at CLN2 disease diagnosis through the BTS program was 3.64 years, which is earlier than the natural history reported average of 5 years. *TPP1* was the highest positive yield gene for autosomal recessive disorders. These findings demonstrate that the use of broad epilepsy gene panel tests can facilitate the earlier diagnosis of CLN2 disease, and simultaneously identify other genetic causes of epilepsy.

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P06.050.C Diagnostic yield and clinical utility of genetic testing in children with seizure onset after two years of age: update over 3-year program in Europe and Middle East

Akashdeep Singh[1], *Kimberly Gall*[2], *Emanuela Izzo*[1], *Kirsi Alakurtti*[2], *Eija H. Seppala*[2], *Lotta Koskinen*[2], *Juha Koskenvuo*[2], *Tero-Pekka Alastalo*[2]

[1]*BioMarin Pharmaceutical Inc, Novato, CA, USA,* [2]*Blueprint Genetics, San Francisco, CA, USA.*

Neurologic and metabolic disorders with epileptic seizures are among the most common genetic disorders presenting in childhood. A molecular diagnosis for patients with epilepsy may allow for etiologically based treatment and management. This program offers genetic testing to patients with epilepsy in Europe and the Middle East. The goals of the program are to determine, in pediatric epilepsy patients between 2-5 years of age, molecular diagnostic yield and the impact on diagnosing neuronal ceroid-lipofuscinosis type 2 (CLN2). CLN2 is a severe, rapidly-progressive neurodegenerative disease with seizure onset at or after 2 years of age. An NGS-based epilepsy panel including Copy number variant (CNV) detection was used. Variant interpretation was performed according to ACMG guidelines. The results from 748 patients with first seizure at or after 24 months, and one additional clinical finding are reported. Median age at testing was 38 months while the median age at first seizure was 27 months. The average delay from first seizure to genetic testing was 9 months. A genetic diagnosis was established in 146 patients for a molecular diagnostic yield of 19.5%. CNVs were reported in 15% of

diagnosed patients and 32% of the CNVs identified were intragenic. The frequent molecular diagnoses included *SCN1A* (n = 17), *MECP2* (n = 13) and *TPP1* (*CLN2*) (n = 12). CLN2 cases received a molecular diagnosis at an average age of 3 years 11 months, 1-2 years earlier than natural history data. At least 93 (63.6%) of diagnosed patients had a disorder that has targeted treatment, evidence for optimizing treatment, or on-going clinical trials.

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P06.051.D Mild neurocognitive and psychosocial outcome despite late-diagnosis of classical phenylketonuria (case report)

Marie-Claire Paulin[1], *Mouna Ben Amor*[2,3], *Lynn Ryan*[2], *Mathieu Mallet*[2], *Marie-Eve Cormier*[2]

[1]*Stan Cassidy Rehabilitation Centre, Horizon Health Network, Fredericton, NB, Canada,* [2]*Centre Hospitalier Universitaire Dr.-Georges-L.-Dumont, Réseau de Santé Vitalité, Moncton, NB, Canada,* [3]*Centre de Formation Médicale du Nouveau-Brunswick, Université de Moncton, Moncton, NB, Canada.*

Introduction: Classical phenylketonuria (PKU) is a genetic condition that impairs the metabolism of phenylalanine and requires lifelong dietary treatment for normal development. PKU is known to cause brain damage and impairment, including moderate to severe intellectual disability, if not treated promptly. Here, we present the case of a late PKU-diagnosed 40-year-old college-educated woman who has mild neurocognitive impairments.

Materials and Methods: Single gene analysis for the *PAH* gene was performed by Next Generation Sequencing. Regular blood-phenylalanine analyses as well as objective and subjective psychometric assessments of intellectual potential, executive function and mental health were conducted.

Results: Genetic analyses revealed compound heterozygous mutations (IVS1+5g>T and p.P281L) in the *PAH* gene. This genotype has been previously reported in patients with classic PKU. Blood phenylalanine levels in our patient ranged from 896 to 2257 µmol/L since diagnosis at the age of 30 (except during her second pregnancy). Despite such toxic levels, this patient only has mild neurocognitive and psychosocial impairments. She does not meet the criteria for even a mild intellectual disability.

Conclusion: Increasingly more cases of untreated or late-diagnosed PKU patients with no major cognitive impairment are reported in the literature. While late-diagnosed PKU is unusual, the diagnosis should be considered in cases of unexplained learning challenges, psychiatric symptoms, or neurocognitive impairment, even if mild, especially if the patient was born during a period or place where neonatal PKU screening was not systematically conducted. More research is needed to explain the variability in the severity of cognitive impairment in certain PKU patients.

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P06.053.B Expanding the clinical spectrum of primary coenzyme Q10 deficiency type 6: the first case with cardiomyopathy

Lisette Leeuwen, *Charlotte M. A. Lubout, Hessel P. Nijenhuis, Linda C. Meiners, Yvonne J. Vos, Johanna C. Herkert*

UMCG, Groningen, Netherlands.

Introduction: Primary coenzyme Q10 deficiency (primary COQ10 deficiency) is a mitochondrial respiratory chain disease caused by biallelic variants in: *COQ2*, *COQ4*, *COQ6*, *COQ7*, *COQ8A*, *COQ8B*, *COQ9*, *PDSS1* or *PDSS2*. The clinical manifestations and age at onset are highly variable. Primary coenzyme Q10 deficiency type 6 (primary COQ10 deficiency-6,MIM#614650) is characterized by steroid-resistant nephrotic syndrome and sensorineural deafness.

Methods: We included a 19-month-old patient with dilated cardiomyopathy. Clinical data of the patient were collected from the medical record. Copy-number variation analysis was performed using SNP-array. Exome sequencing (ES) was performed using a parent-offspring trio approach. Literature search was performed(Pubmed).

Results: Our patient presented with cardiorespiratory insufficiency due to dilated cardiomyopathy at age 19 months. Additionally, she was found to have proteinuria, microcephaly and mild developmental delay. There was no history of hearing loss, vision problems, movement disorder, or epilepsy. Parents were consanguineous (second cousins). Family history was negative for cardiac diseases. SNP-array was normal. Whole ES revealed a homozygous pathogenic variant, c.763G>A,p.(Gly255Arg), in *COQ6*, for which both parents were heterozygous. Primary COQ10 deficiency-6 has previously been reported in 29 patients but cardiomyopathy has not been reported. Although not reported in primary COQ10 deficiency-6, cardiomyopathy has been described in 26 patients with other types of primary COQ10 deficiency.

Conclusions: This case expands the clinical spectrum of primary COQ10 deficiency-6 and underscores the importance of screening for multiple system disease, including cardiac evaluation, in these patients. Genes involved in primary COQ10 deficiency, including *COQ6*, should be included in gene panels for pediatric cardiomyopathy.

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P06.054.C Rare types of the mutations cause pyruvate carboxylase deficiency in 2 patients

Polina Tsygankova[1], *Nikita Beskorovainiy*[1], *Marina Minzhenkova*[1], *Vyacheslav Tabakov*[1], *Lyudmila Bessonova*[1], *Vera Zarubina*[2], *Natalia Pechatnikova*[2], *Marina Kurkina*[1], *Igor Bychkov*[1], *Ekaterina Zakharova*[1]

[1]*Research centre for medical genetics, Moscow, Russian Federation,* [2]*Morozov Children's City Clinical Hospital, Moscow, Russian Federation.*

Introduction: Pathogenic variants in pyruvate carboxylase (PC) gene cause a wide spectrum of recessive phenotypes, ranging from the early fatal encephalopathy to the adult benign form. Clinical presentation of the PC-deficiency is not specific, frequent mutations are not described for the *PC* gene, thus, the whole

exome (WES) and genome (WGS) sequencing could help to find rare cases of this metabolic disease.

Results: Two patients were suspected for having pyruvate metabolism disorder based on the urine organic acids profile and clinical picture. In a patient 1, 6 y.o. o boy with ataxia, hypoglycemia, episodes of lactic acidosis WGS showed heterozygous missense variant c.1372A>G; p.(Asn458Asp) in the *PC* gene and the loss of heterozygosity on *PC* cDNA. Additional bioinformatic analysis with Manta tool revealed soft-clipped reads mapped to chromosomes 11 and 1, so a reciprocal translocation which disrupts the 5'prime end of the *PC* gene including exons 1 and 2 was proposed. The translocation was validated via FISH-analysis and PCR with flanking primers. The segregation was confirmed. The second patient, 13 y.o. girl with psychomotor delay, hepatopathy, episodes of lactic acidosis, ketonuria, had novel homozygous intronic variant c.1983-116C>T revealed on WES. The cDNA analysis from blood cells established three aberrant mRNA isoforms due to exonization of intron 17 sequences in the vast majority of *PC* transcripts.

Conclusion: After routine gene sequencing in case of suspicion of PC-deficiency WES and WGS with deep bioinformatic analysis could be needed.

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P06.055.D SLC25A42-associated mitochondrial encephalomyopathy report of additional founder cases and functional characterization of a novel deletion

Mazhor Salem Aldosary[1], *Shahad Baselm*[1], *Maha Abdulrahim*[1], *Rawan Al-Mass*[1], *Maysoon Alsagob*[2], *Zainab AlMasseri*[1], *Rozeena Huma*[1], *Laila AlQuait*[1], *Tarfa Al-Shidi*[1], *Eman Al-Obeid*[1], *Albandary AlBakheet*[1], *Basma Alahideb*[1], *Lujane Alahaideb*[1], *Alya Qari*[1], *Robert Taylor*[3], *Dilek Colak*[1], *Moeenaldeen AlSayed*[1], *Namik Kaya*[1]

[1]*King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia,* [2]*King Abdulaziz City for Science and Technology, Riyadh, Saudi Arabia,* [3]*Wellcome Centre for Mitochondrial Research, Riyadh, Saudi Arabia.*

SLC25A42 is the main transporter of Coenzyme A into mitochondria. To date, 15 subjects are reported due to one of two, bi-allelic homozygous missense variants in the *SLC25A42* as a cause of mitochondrial encephalomyopathy; 14 subjects are of Saudi origin and share the same founder variant, c.871A>G:p.Asn291Asp. The remaining singleton is German carrying a variant at canonical splice site, c.380+2T>A. In this study, we describe the clinical manifestations and disease course in an additional six Saudi patients whose blood samples were assessed by Affymetrix's axiom autozygosity mapping, and whole exome sequencing. After a comprehensive filtering process, two variants of *SLC25A42* were confirmed with Sanger sequencing and fully segregated in the tested family members. While five patients have the Saudi founder p.Asn291Asp variant, one subject has a novel deletion. Functional analyses on fibroblasts obtained from this patient revealed that the deletion causes significant decrease in mitochondrial oxygen consumption and ATP production compared to healthy individuals. Moreover, extracellular acidification rate revealed significantly reduced glycolysis, glycolytic capacity, and glycolytic reserve as compared to controls. There were no changes in the mitochondrial DNA content of patient fibroblasts. Immunoblotting revealed significantly diminished protein expression due to the deletion. Our study expands the molecular spectrum of this condition and provides further evidence of mitochondrial dysfunction as a central cause of pathology. Finally, this disorder should be included in the differential diagnosis of any patient with unexplained motor and speech delay, recurrent encephalopathy with metabolic acidosis, intermittent or persistent dystonia, lactic acidosis, and basal ganglia lesions.

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P06.056.A A recessive mutation in *TFAM* causes mtDNA depletion associated with primary ovarian insufficiency, seizures, and hearing loss

Farid Ullah[1,2], *Waqar Rauf*[1], *Kamal Khan*[2], *Sheraz Khan*[1], *V Oliveira*[3], *K Bell*[4], *M Tariq*[1], *S Bakhshalizadeh*[4], *T Philippe*[5], *A Sinclair*[4], *E Tucker*[4], *S He*[6], *S M. Baig*[1], *E E. Davis*[7]

[1]*National Institute for Biotechnology and Genetic Engineering (NIBGE-C), Faisalabad; Pakistan Institute of Engineering and Applied Sciences (PIEAS), Islamabad, Pakistan,* [2]*Stanley Manne Children's Research Institute, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL, USA, Chicago, IL, USA,* [3]*Department of Veterinary Medicine, Faculty of Animal Science and Food Engineering, University of São Paulo, Pirassununga, São Paulo, Brazil,* [4]*Murdoch Children's Research Institute, Royal Children's Hospital and Department of Pediatrics, University of Melbourne, Melbourne VIC, Melbourne, Australia,* [5]*Department of endocrinology and reproductive medicine, center for rare endocrine and gynecological diseases, Sorbonne Université Pitié Salpétrière Hospital, Paris, France,* [6]*BGI-Shenzhen, Shenzhen, China,* [7]*Stanley Manne Children's Research Institute, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, Chicago, IL, USA.*

Mitochondrial diseases are common, genetically heterogeneous disorders in both pediatric and adult populations. They are caused by defects in the processes of oxidative phosphorylation, apoptosis, and failure of essential bioenergetic supply to mitochondria. TFAM (transcription factor A, mitochondrial) is a key component of the mitochondrial replisome machinery that maintains mtDNA transcription and replication. Here, we present two affected individuals from a consanguineous Pakistani pedigree that presented with primary ovarian insufficiency (POI) and seizures. We performed whole exome sequencing on parents and affected siblings and found a recessive missense variant in *TFAM*, c.694C>T (p.Arg232Cys), that segregated with disease. Notably, this DNA change is identical to a recently reported sporadic case of similar ethnic origin who displays POI, seizures, and sensorineural hearing loss. In patient derived skin fibroblasts, we observed depletion of mtDNA and significant alteration in the respiratory function and morphology of mitochondria. Moreover, we observed reduced numbers of nucleoids with significant changes in nucleoid size or shape in patient cells compared to matched controls. Next, we investigated the effect of *tfam* loss in a zebrafish model. Knock out (KO) mutants recapitulate the mtDNA depletion and ovarian dysgenesis reminiscent of patient phenotypes. Together, our genetic and functional data indicate that TFAM plays a pivotal role in the development of gonads. Thus, our findings expand the list of mitochondrial disease phenotypes and helps guide disease management and diagnosis

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P06.057.B Circulating DNA methylation biomarkers for type 2 diabetes diagnosis from saliva

Manuela Hofner[1], *Ulrike Kegler*[1], *Klemens Vierlinger*[1], *Anja Buhmann*[1], *Michael Leutner*[2], *Walter Pulverer*[1], *Alexandra Kautzky-Willer*[2], *Christa Noehammer*[1]

[1]*AIT Austrian Institute of Technology GmbH, Molecular Diagnostics Unit, Vienna, Austria,* [2]*Medical University of Vienna, Clinical Division of Endocrinology and Metabolism, Vienna, Austria.*

Introduction: Saliva is a readily and repeatedly available body fluid, which can be obtained via non-invasive, painless collection. The aim of this study was to define salivary extracellular vesicle-derived epigenetic biomarkers suitable for type 2 diabetes diagnosis.

Methods: After evaluation of several isolation kits for saliva-derived extracellular vesicles we performed a proof of principle study, comparing DNA methylation profiles in saliva and serum extracellular vesicles from healthy individuals. For our type 2 diabetes DNA methylation discovery study cell-free saliva was collected from a diabetic patient cohort (type 2 diabetes, pre-diabetes, gestational diabetes, healthy) and extracellular vesicles thereof prepared. Genome-wide DNA methylation profiling was performed using extracellular vesicle-derived DNA on Illumina EPIC arrays.

Results: After having identified the best suited method for extracellular vesicle isolation from saliva we could successfully show the feasibility of using saliva as a potential diagnostic matrix in our comparative profiling study, as the DNA methylation profiles in healthy probands showed a big overlap between serum- and saliva-derived extracellular vesicles. In addition, we were able to successfully run a type 2 diabetes genome-wide DNA methylation discovery study from salivary extracellular vesicle-derived DNA and identified potential DNA-methylation based candidate biomarkers.

Conclusions: This study once more demonstrated saliva to be a most promising sample matrix for disease diagnostics. Furthermore genome-wide profiling technologies such as methylation bead arrays could be successfully applied to cell-free body fluids despite low amounts of circulating DNA present there.

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P06.058.C VARS2-linked mitochondrial disease - an emerging phenotypic spectrum

Raquel Gouveia, *Márcia Rodrigues, Oana Moldovan, Ana Berta Sousa*

Serviço de Genética Médica, Hospital de Santa Maria, Centro Hospitalar Universitário Lisboa Norte, Lisboa, Portugal.

Introduction: Bi-allelic variants in *VARS2*, a nuclear gene coding for valyl-tRNA synthetase, cause autosomal recessive combined oxidative phosphorylation deficiency type 20 (*MIM#609060*), characterized by a variable combination of mitochondrial encephalopathy, developmental delay, hypotonia, epilepsy, cardiomyopathy and structural brain anomalies, usually with a neonatal onset and severe disease course.

Case Report: Family 1: A 23-year-old man, who was healthy until age 11, presented with generalized tonic-clonic seizures, evolving with persistent intentional tremor, and mild learning disability. He had an elder sister with laryngomalacia requiring tracheostomy in childhood, and late-onset epilepsy; and a younger brother with cerebral palsy after cardiac arrest at 3 months, hypotonia, and hyperlactacidemia. They both had hypertrophic cardiomyopathy (HCM), which resolved spontaneously. All three siblings had bilateral basal ganglia calcifications.Family 2: A female neonate, with a prenatal diagnosis of HCM, died at 40 days from hyperlactacidemia and severe HCM. She was the first child of non-consanguineous parents. Her mother was again pregnant, and a fetal echocardiogram had been suggestive of right ventricular hypertrophy.

Results: Reanalysis of WES data from case 1 revealed two compound likely pathogenic heterozygous variants in *VARS2* gene: c.1258G>A, p.(Ala420Thr) and c.1100C>T, p.(Thr367Ile). Combined heterozygosity for two variants in *VARS2* was also identified in case 2 by clinical exome sequencing: c.1258G>A, p.(Ala420Thr) and c.1079C>T, p.(Ala369Val). Targeted analysis confirmed the fetus was affected.

Conclusions: These cases illustrate the intra- and extra-familial spectrum of variability associated with *VARS2* variants, including milder phenotypes that complicate genetic counselling, especially in the prenatal setting.

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P06.059.D Functional analysis of the PCCA and PCCB genes variants, predicted to affect splicing

Igor Bychkov, ***Artur Galushkin****, Aleksandra Filatova, Andrey Nekrasov, Marina Kurkina, Galina Baydakova, Alexandra Ilyushkina, Mikhail Skoblov, Ekaterina Zakharova*

Research centre for medical genetics, Moscow, Russian Federation.

Introduction: Mutations in the PCCA and PCCB genes result in propionic acidemia (PA) - rare autosomal recessive metabolic disease. In most cases the symptoms of PA manifest in the early neonatal period and without treatment quickly become life-threatening. Thus, the fast and proper genetic diagnosis plays an important role in patients` management. During the analysis of public repositories of PA mutations, we faced the problem of incorrect classification of splicing variants, which is inconsistent with ACMG/AMP recommendations. Also, there is a part of variants, which are classified as missense or synonymous variants of uncertain significance, being highly spliceogenic.

Materials and methods: After analyzing of ClinVar and HGMD databases for PA mutations 23 variants were selected, some of which were located in splicing sites, but lack the functional characterization at mRNA level and those, classified as missense or synonymous, but were bioinformaticaly predicted as spliceogenic. To characterize the effect of these variants at mRNA level the minigene assay was performed.

Results: We characterized the splicing alterations for 14 variants (PCCA:c.183+4del4, c.468+1G>A, c.717-2A>G, c.1187T>G; (p.Val396Gly), c.1284+2dup, c.1353+5delG, c.1353+5_1353+9del, c.1643+1_1643+2dup, c.1899+2_1899+3insCT, c.2040G>A; (p.Ala680=), c.2119-9A>G; PCCB: c.543G>C; (p.Leu181=), c.655-2A>G, c.1091-8_1091-3del). For the cases where the splicing outcome didn't disrupt the mRNA reading frame, we characterized the affected structures on a protein level.

Conclusion: The performed functional analysis allowed us to characterize the splicing outcome for 14 PA variants and reclassify them according to ACMG/AMP guidelines.

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P07 Immunology and Hematopoietic System

P07.001.A Molecular basis of thalassemia in Dobrogea, Romania

Anca Mitroi[1,2], *Costel Brinzan*[1,2], *Mariana Aschie*[1], *Adriana Apostol*[1], *Gerogeta Cozaru*[1,2]

[1]*Emergency Clinical County Hospital of Constanta, Constanta, Romania,* [2]*Ovidius University, Constanta, Romania.*

Introduction: Thalassemia is a group of hemoglobin disease caused by a reduction or absence in the synthesis of beta or alpha globins chain that results in a group of hereditary anemia of varying clinical severity.

Material and methods: 71 patients laboratory suspected with thalassemia were studied for characterization with as either heterozygous or homozygous for beta and alpha thalassemia. Molecular analysis was done by PCR and reverse-hybridization in order to detect 22 of mutations most commonly associated with beta-thalassemia (Mediterranean type) and 21 of mutation covering >90% of α-globin defect.

Results: 28% of cases have mutations of alpha or beta globins gene and only 4% of cases have concomitant mutation in alpha and beta globins gene. 13% of patients have mutation for alpha thalassemia represented by: $-\alpha^{3.7}$gene deletion (6%), $\alpha\alpha\alpha^{3.7}$ gene triplication (6%) and α2polyA-2(1%). Only one patient was homozygous for $-\alpha^{3.7}$gene deletion. 19% of patient of patients have mutation for beta thalassemia represented by: IVS1.100 [G>A] (6%), IVS 2.745[C>G] (6%), IVS 1.6[T>C] (4%), cd8[-AA](2%) and IVS2.1[G>A](1%). Only one patient was homozygous for IVS 1.6[T>C].

Conclusion: In our study the most frequent mutation for beta thalassemia was IVS1.100 [G>A] and IVS 2.745[C>G] similar with other studies. The mutations cd8[-AA] and IVS2.1[G>A] were not identified in former studies. Regarding mutation responsible for alpha thalassemis there are not other published studies in Romania.

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P07.003.C Genetic diagnoses in paediatric rheumatology clinic

Hannah L. Titheradge, *Eslam Al-Abadi*

Birmingham Women's and Children's NHS Foundation Trust, Birmingham, United Kingdom.

Introduction: Patients within paediatric rheumatology clinics commonly have features of systemic inflammatory disease. This is more commonly multifactorial in nature, however increasingly monogenic diagnoses are made. An accurate diagnosis is important to optimise patient management.

Method: We review the outcomes from the first 3 years of a newly formed paediatric rheumatology and genetic multidisciplinary clinic. Where indicated genomic testing was performed to try to identify a monogenic cause for the patient's clinical problems. Patients seen had diagnoses of SLE, periodic fever syndrome and atypical juvenile idiopathic arthritis in the main.

Results: We review the diagnoses made from genomic testing in this patient group and the impact on management. Patient diagnoses have included monogenic auto-inflammatory disease, complement deficiency, Fabry disease, XXX syndrome, camptodactyly-arthropathy-coxa vara-pericarditis syndrome and arthropathy secondary to tufting enteropathy.

Conclusion: This highlights the importance of a genetic diagnosis, which can positively influence patient management and also significantly influences family planning discussions for the patient and their families.

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P07.004.D Diagnostic performance of NGS panel genetic testing in patients suspected for autoinflammatory syndrome

Gorjan Milanovski[1], *Meri Kirijas*[1], *Boban Dobrevski*[1], *Teodora Brnjarchevska Blazevska*[1], *Olivija Efinska Mladenovska*[1], *Olgica Sibinovska*[1], *Todor Arsov*[1], *Katarina Stavrikj*[2], *Kristina Mironska*[2], *Beti Gjurkova*[2], *Aleksandar Petlichkovski*[1]

[1]*Ss. Cyril and Methodius University in Skopje, Faculty of Medicine-Skopje, Institute of Immunobiology and Human Genetics, Skopje, Macedonia, The Former Yugoslav Republic of,* [2]*Ss. Cyril and Methodius University in Skopje, Faculty of Medicine-Skopje, PHI University Clinic for child diseases, Skopje, Macedonia, The Former Yugoslav Republic of.*

Introduction: Autoinflammatory syndrome (AIS) constitutes a heterogenous group of disorders defined by recurrent episodes of systemic inflammation in the absence of pathogens, autoantibodies and/or self-reactive lymphocytes. We report initial results from our experience with NGS gene panel testing for AIS.

Materials and methods: Nineteen pediatric and four adult patients with clinical suspicion of AIS were referred for genetic testing. DNA samples were analyzed on Ion Torrent platform, using an AmpliSeq AIS gene panel, which included 34 genes (*ASAH1, CARD14, DDX58, ELANE, IFIH1, IL10RA, IL10RB, IL1RN, IL36RN, LPIN2, MEFV, NLRC4, NLRP12, NLRP3, NOD2, MVK, PLCG2, PSMB8, SAMHD1, RBCK1, SLC29A3, RNASEH2B, ADAR, RNASEH2A, RNASEH2C, TNFAIP3, TNFRSF11A, TNFRSF1A, NLRP1, OTULIN, TMEM173, HAX1, PSTPIP1, TREX1)*. Variant calling and interpretation of pathogenicity was performed using the IonReporter v.5.14 variant analysis software and the ACMG criteria.

Results: The diagnostic yield of our gene panel in 23 unrelated patients was about 10% - 2/23 patients had a likely pathogenic variant, both in the *MEFV* gene (c.2084A>G and c.2282G>A) associated with familial Mediterranean fever. A "significant" VUS variant in a gene consistent with the clinical phenotype was identified in additional 10% of cases, one in *NLRP12* gene (c.1054C>T) and *TNFRSF1A* (c.434A>G).

Conclusion: These findings are comparable with those from the literature and support the use of this type of genetic testing in AIS, as it helps in establishing the diagnosis of these difficult to diagnose and rare conditions.

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P07.005.A Relationship between *BCR-ABL1* FISH patterns, clonal evolution, and response in CML patients in Bosnia and Herzegovina

Erna Islamagic[1], *Sabira Kurtovic*[2], *Azra Hasic*[1], *Amina Kurtovic-Kozaric*[3]

[1]*Faculty of Science, Sarajevo, Bosnia and Herzegovina,* [2]*Hematology Clinic, Clinical Center of the University of Sarajevo, Sarajevo, Bosnia and Herzegovina,* [3]*Clinical Pathology, Cytology and Human Genetics, Clinical Center of the University of Sarajevo, Sarajevo, Bosnia and Herzegovina.*

Introduction: *BCR-ABL1* FISH was part of routine diagnostic procedure for chronic myeloid leukaemia (CML) patients; however, prognostic impact of the various signal patterns has not been widely analysed. The aim of the study was to correlate different *BCR-ABL1* FISH pattern types to survival probabilities and response in CML patients.

Materials and Methods: In this study, FISH results and clinical data for CML-CP patients (n = 83) treated in the period 08/2005-12/2019 in the Federation of Bosnia and Herzegovina were evaluated. Patients were categorised based on the pattern type into Group 1 (single patterns 1R1G2F, 1R1G1F, 2R1G1F, 1R2G1F, n = 71) and Group 2 (complex patterns, n = 12). Patients' variables included TKI treatment (imatinib and/or nilotinib), OS, cytogenetic (CCyR) and molecular responses (MMR, MR^4, and MR^5). Survival probabilities were estimated using the Kaplan-Meier method (log-rank test).

Results: We analysed 83 patients with median follow-up of 76.5 months. Patients with single and complex patterns were on imatinib (45% vs. 33%), nilotinib (20% vs. 8%), switched from imatinib to nilotinib (24% vs. 59%), or did not receive TKI therapy (11% vs. 0%). At 60 months, OS was significantly different between Group 1 and Group 2 (83% vs. 25%, p = 0.012). Additionally, CCyR rates at 60 months were 76% vs. 0% (p = 0.013) and MMR rates were 72% vs. 0% (p = 0.042). No significant differences were found in achievement of MR^4 or MR^5 in analysed groups (p = 0.107 and p = 0.256, respectively).

Conclusions: Our study showed that complex *BCR-ABL1* FISH patterns were associated with worse response and progression in CML patients in Bosnia.

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P07.007.C Identification of the molecular etiology in congenital hemolytic anemias beyond hemoglobinopathies using a targeted next-generation sequencing panel

Tahir Atik[1], *Esra Isik*[1], *Yesim Aydinok*[1], *Mehmet Fatih Orhan*[2], *Yesim Oymak*[3], *Zeynep Karakas*[4], *Nazan Sarper*[5], *Canan Albayrak*[6], *Nihal Karadas*[1], *Ibrahim Eker*[7], *Selma Unal*[8], *Ozgur Cogulu*[1], *Ferda Ozkinay*[1]

[1]*Ege University Faculty of Medicine, Department of Pediatrics, Izmir, Turkey,* [2]*Sakarya University Faculty of Medicine, Department of Pediatrics, Sakarya, Turkey,* [3]*Dr. Behcet Uz Children's Hospital, Division of Pediatric Hematology, Izmir, Turkey,* [4]*Istanbul University Faculty of Medicine, Department of Pediatrics, Istanbul, Turkey,* [5]*Kocaeli University Faculty of Medicine, Department of Pediatrics, Kocaeli, Turkey,* [6]*Ondokuz Mayis University Faculty of Medicine, Department of Pediatrics, Samsun, Turkey,* [7]*Afyon Kocatepe University Faculty of Medicine, Department of Pediatrics, Afyon, Turkey,* [8]*Mersin University Faculty of Medicine, Department of Pediatrics, Mersin, Turkey.*

Aim: Congenital hemolytic anemias (CHA) are a group of disorders showing genetic heterogeneity caused by mutations in genes encoding proteins involved in the production or structure of erythrocytes. Despite advances in next generation sequencing technologies, approximately 60% of patients with CHA can be molecularly diagnosed. This study aims to evaluate the utility of a targeted NGS panel in CHA.

Method: Eighty-seven CHA cases from 84 families referred to our center for molecular analysis were enrolled in the study. Participating physicians recorded patients' demographics, family history, and laboratory test results onto the database after receiving informed consent from the patients/legal representatives. Molecular analysis was performed using an NGS panel including 4811 genes (TruSight One Disease® Panel by Illumina). In the panel, 66 genes associated with CHA were evaluated concerning diagnostic compatibility with the patients' clinical and laboratory presentation.

Results: The molecular diagnostic rate of this targeted NGS panel of 66 genes was 62% (54 of 87 cases). In the 54 cases having a molecular diagnosis, 57.4% (n = 31) were diagnosed with red cell membrane protein defects and 42.6% (n = 23) with enzyme deficiencies. We found 48 different disease-causing variants in 12 genes (ANK1, SPTB, SPTA1, SLC4A1, EPB41, ABCB6, AK1, BPGM, G6PD, HK1, and PKLR). Twenty-three of the variants were novel.

Conclusion: In this study, we achieved a high molecular diagnostic rate. A targeted NGS panel provides a correct and definitive diagnosis of patients with CHA. It is essential for the appropriate treatment of those patients.

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P07.010.B NGS-based diagnosis of congenital erythrocytosis: extended targeted NGS and identification of novel candidate genes

Aleša Kristan[1], *Jana Tomc*[1,2], *Tadeja Režen*[2], *Tanja Kunej*[3], *Rok Količ*[4], *Andrej Vuga*[4], *Martina Fink*[5], *Saša Anžej Doma*[5], *Irena Preložnik Zupan*[5,6], *Tadej Pajič*[5,7], *Nataša Debeljak*[1]

[1]*Medical Centre for Molecular Biology, Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia,* [2]*Centre for Functional Genomics and Bio-Chips, Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia,* [3]*Department of Animal Science, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia,* [4]*Kemomed Research and Development, Kemomed Ltd, Kranj, Slovenia,* [5]*Clinical Department of Hematology, University Medical Centre Ljubljana, Ljubljana, Slovenia,* [6]*Department of Internal Medicine, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia,* [7]*Clinical Institute of Genomic Medicine, University Medical Centre Ljubljana, Ljubljana, Slovenia.*

Introduction: Congenital erythrocytosis is a rare haematological disorder with abnormally high erythrocyte count. The diagnostics is highly challenging due to the heterogenic genetic background. Patients are usually screened for variants in nine genes involved in erythropoiesis and oxygen homeostasis regulation, however in only 30% the genetic cause is identified. Our aim was to extend current genetic testing of patients with idiopathic erythrocytosis and to discover new disease-driven pathways and genes.

Material and Methods: Targeted NGS covering 24 erythrocytosis and 15 haemochromatosis-associated genes was performed on 40 patients, selected based on the national diagnostic algorithm for erythrocytosis. Additional disease-driven mechanisms were explored through a review of the literature and several tools, including Reactome, String and GeneCards.

Results: Targeted NGS identified the pathogenic variant in one patient, c.1609G>A (p.Gly537Arg) in the *EPAS1* gene, responsible for the development of hereditary erythrocytosis type 4. Genetic screening of other patients revealed several VUS below 1% in the European population, that could be the single cause for erythrocytosis or could have a combine effect. In silico we identified several new pathways, containing genes involved in epigenetic modifications, sumoylation and nuclear-cytoplasmic shuttling, which might influence the mechanisms of disease development.

Conclusion: Targeted NGS revealed one pathogenic variant and several VUS, that require further functional assessment. However,

the inclusion of novel genes in current diagnostic solutions or broader approaches, like WGS or WES, are needed for prompt and accurate diagnosis of congenital erythrocytosis. ARRS grant L3-9279, P1-0390 and Young Researcher funding.

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P07.011.C A case report of an atypical *FIP1L1-PDGFRA* fusion in a patient with hypereosinophilia

Sadiye Ekinci[1], *Güldane Cengiz Seval*[2], *Halil Gürhan Karabulut*[1], *Arzu Vicdan*[1], *Seher Yüksel*[3], *Işınsu Kuzu*[3], *Günhan Gürman*[2], *Timur Tuncalı*[1]

[1]*Department of Medical Genetics, Ankara University Faculty of Medicine, Ankara, Turkey,* [2]*Department of Hematology, Ankara University Faculty of Medicine, Ankara, Turkey,* [3]*Department of Pathology, Ankara University Faculty of Medicine, Ankara, Turkey.*

Introduction: *FIP1L1-PDGFRA* fusion, which originates from an interstitial deletion in 4q12, is observed in diverse eosinophilia-associated hematologic disorders. In FIP1L1-PDGFRA fusion protein, the JM domain of PDGFRA that is known to serve as an autoinhibitory function is deleted and the tyrosine kinase region comes under the control of FIP1L1 promoter causing a constitutive kinase activation. Here, we present a case with hypereosinophilia in whom an atypical *FIP1L1-PDGFRA* fusion pattern in bone marrow specimen was detected. The case was a 77 years-old-male with hypereosinophilia for four years and with mild cutaneous symptoms. In pathological examination, it was confirmed that there was no bone marrow infiltration that might be seen in secondary eosinophilia and in other hematological neoplasms. Two previous analyses from peripheral blood samples failed to show the existence of FIP1L1-PDGFRA fusion.

Materials and Methods: Reverse transcriptase-polymerase chain reaction (RT-PCR) was used for the detection of *FIP1L1-PDGFRA* rearrangement and the result was confirmed by sanger sequencing.

Results: Direct sequencing of the RT-PCR product revealed that normally spliced FIP1L1 exon 10 fused with PDGFRA sequence following the genomic breakpoint within PDGFRA exon 12. Interestingly, an-inframe deep intronic sequence of 30 bp derived from FIP1L1 intron 10 was observed between the two genes in the fusion transcript.

Conclusions: For cases in which the fusion cannot be detected from the peripheral blood, bone marrow analysing should be considered, given the fact that patients with FIP1L1-PDGFRA fusion benefit from imatinib treatment.

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P07.012.D MEFV gene mutation frequency in Georgian FMF patients

Dodo Agladze[1,2,3], *Lali Margvelashvili*[2], *Saba Iordanishvili*[4], *Maka Ioseliani*[5], *Teimuraz Mikeladze*[6], *Hasmik Hayrapetyan*[7], *Tamara Sarkisian*[7], *Oleg Kvlividze*[3]

[1]*Research Institute of Clinical Medicine, Tbilisi, Georgia,* [2]*Pediatric Surgery Center KidCo, Tbilisi, Georgia,* [3]*School of Medicine, New Vision University, Tbilisi, Georgia,* [4]*Petre Shotadze Tbilisi Medical Academy, Tbilisi, Georgia,* [5]*New Hospitals, Tbilisi, Georgia,* [6]*Caucasus Medical Center, Tbilisi, Georgia,* [7]*Center of Medical Genetics and Primary Health Care, Yerevan, Armenia.*

Introduction: Familial Mediterranean Fever (FMF) is an autosomal recessive hereditary disorder caused by mutation in *MEFV* gene. Higher prevalence has been estimated in populations with Mediterranean ancestry (Armenian, Arab, Italian, Jewish, Greek and Turkish). Prevalence of FMF has not been assessed in Georgian population; however, it is estimated to be high since Georgia borders Turkey and Armenia. The study aims to analyze the frequency of *MEFV* mutations in individuals suspected for FMF and determination of *SAA1* polymorphism in diagnosed patients, in order to estimate FMF prevalence in Georgia.

Material and methods: 118 Georgian individuals suspected for FMF were screened for *MEFV* gene mutation variants M694V, V726A, M680I(G/C), M680I(G/A), F479L, E148Q, M694I, R761H, P369S, 1692del, K695R, A744S using PCR methods. *SAA1* polymorphism was analyzed in confirmed patients.

Results: From 118 patients, FMF cases were confirmed in 45 (38.1%), while 22(18.6%) carried a single *MEFV* mutation. From 112 alleles with detected mutations, frequency distribution of *MEFV* mutations is as follows: M694V 56.3%, M680I(G/C) 11.6%, V726A 10.7%, E148Q 7.1%, R761H 6.3%, M694I 3.6%, F479L 2.7%, K695R 1.8%. 4 variants(M680I(G/A), P369S, 1692del and A744S) were not detected. From affected 45 individuals, homozygous forms were detected in 33.3% (M694V 31.1%, M680I(G/C) 2.2%) of the cases. 5 affected individuals (11.1%) showed α/α polymorphism of *SAA1* gene.

Conclusion: Based on study results the most frequent *MEFV* gene mutation is M694V. Mutation frequency distribution of *MEFV* gene in Georgian patients is similar to Armenian and Turkish populations. This suggests the importance of prevalence analysis of FMF in Georgian population.

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P07.014.B A novel variant in *SERPING1* is associated with a recessive form of hereditary angioedema in a consanguineous Brazilian family

Luana Sella Motta Maia[1], *Fernanda Leonel Nunes*[2], *Marina Mendonça Dias*[2], *L. Karla Arruda*[2], *Bettina Burger*[1], *Sven Cichon*[1,3]

[1]*University of Basel and University Hospital Basel, Basel, Switzerland,* [2]*University of São Paulo, Ribeirão Preto, Brazil,* [3]*Institute of Neuroscience and Medicine (INM-1), Jülich, Germany.*

Introduction: Hereditary angioedema (HAE) is characterized by recurrent episodes of severe and often life-threatening, non-pruritic subcutaneous and submucosal edema. Different clinical and genetic subtypes exist, and the most common forms (HAE types I/II) are caused by dominant variants in the *SERPING1* gene, resulting in C1-inhibitor (C1-INH) deficiency. C1-INH is a multifunctional serine protease inhibitor (serpin) that controls proteases in multiple important plasmatic pathways including inflammation. Its deficiency leads to uncontrolled activation of the kinin-kallikrein system resulting in an extended generation of bradykinin.

Materials and Methods: Two sisters from consanguineous, unaffected parents were severely affected by HAE since adolescence (13 yrs.) and young adulthood (28 yrs.). No other member of this large, Brazilian family presented any symptoms of HAE. We extracted gDNA from whole blood of 34 family members and sequenced the coding region of the *SERPING1* gene.

Results: In both symptomatic sisters, we found a homozygous missense variant in exon 6 (c.964G>A), resulting in an amino acid

exchange (p.Val322Met). Fourteen family members (including the sisters' parents) were heterozygous carriers of the variant. Careful clinical re-examination of these individuals excluded any HAE symptoms.

Conclusions: HAE I/II typically is an autosomal dominant condition caused by more than 600 described *SERPING1* variants, and there are only very few reports of HAE variants acting in a recessive fashion. The novel recessive variant identified in several members of our Brazilian family gives us the unique opportunity to study the functional effect of the C1-INH p.Val322Met variant on the control of the kinin-kallikrein system.

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P07.015.C Two new cases diagnosed with Hermansky-Pudlak Syndrome

Ceren Alavanda[1], *Esra Arslan Ates*[2], *Bilgen Bilge Geckinli*[1], *Senol Demir*[2], *Hamza Polat*[1], *Fatma Uguzdogan*[1], *Mehmet Ali Soylemez*[1], *Pinar Ata*[1], *Ahmet Arman*[1]

[1]*Marmara University School of Medicine, Medical Genetics Department, Istanbul, Turkey,* [2]*Marmara University Pendik Research and Training Hospital, Medical Genetics Department, Istanbul, Turkey.*

Hermansky-Pudlak syndrome (HPS) is a rare autosomal recessive pleiotropic disease. Its predicted prevalence is 1-9/1.000.000. It is characterized with oculocutaneous albinism(OCA), bleeding diathesis(BD), and inflammatory bowel disease(IBD). Among ten different subtypes, HPS-1 is the most common and severe form. Pulmonary fibrosis can be seen in HPS-1, HPS-2, and HPS-4 patients. HPS-related gene products take part in BLOCs (biogenesis of lysosome-related organelle complexes) and are important for biogenesis of melanosome and platelet dense granules. Thirty-seven and fourty-nine-year-old unrelated male patients with the clinical diagnosis of HPS were referred to our clinic. Their parents both had consanguineous marriages. Younger patient had OCA, BD, nystagmus and IBD. Additionally, he had mental retardation (MR) and renal failure. He had no history of lung disease. A-CGH was performed due to MR, which is an unexpected finding in HPS, and was found to be normal. Older patient also had OCA, BD, nystagmus and IBD. Unlike the first patient, this patient had normal intelligence and was diagnosed with pulmonary fibrosis at the age of 44. HPS-related genes were sequenced at Illumina NovaSeq Platform. Homozygous c.972delC (p.Met325Trpfs*6) and c.1189delC (p.Gln397Serfs*2) mutations in the *HPS1* gene were detected in the first and second patient, respectively. Both were recurrent mutations, previously associated with HPS type 1. Herein we report clinical and genetic findings of two patients with HPS. Although HPS is rare syndrome clinical findings are typical for diagnosis. Identifying the subtype with molecular studies is important for patient's follow-up. Our patients will contribute the phenotype-genotype correlations in this syndrome.

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P07.016.D Analysis of IL2, IL10 genes polymorphisms in seronegative to herpesviruses children with recurrent respiratory infection

Yulia Makarova, *Angela Alexandrova, Elena Mashkina, Olga Lyangasova*

Southern Federal University, Rostov-on-Don, Russian Federation.

Background: Features of the clinical course and the persistence of herpesvirus infections are associated with both the heterogeneity of the pathogen and the person immune status. Cytokine genes and their SNPs affect the resistance to herpesvirus infections and features of the viral persistence. In the current work, we investigated the SNPs of the *IL10, IL2* and their association with the immune status of recurrent respiratory infection (RRI) children.

Material and methods: DNA samples isolated from the peripheral blood of 76 RRI children were used. ELISA was used for detection of IgG to CMV and EBV. Allele-specific PCR was used to analyze the rs2069762 and rs1800896. The method of flow cytometry was used to determine different types of lymphocytes.

Results: Significant differences in the frequencies of genotypes for the polymorphism *-1082G>A* of the *IL10* gene between seronegative children and children with IgG to EBV were found. The genotype *-1082AA IL10* was dominated in group of seronegative children. A direct correlation was established between the number of B cells and the GG genotype of the IL10 gene in children with EBV and CMV (<0,05). The inverse correlation was found between the GG genotype and the number of NK cells in children with EBV and CMV (<0,05).

Conclusion: Features of the genotype for cytokine genes can affect the ratio of different classes of the immune system cells and, accordingly, the formation of immunity to herpes viruses. This study was funded by the Ministry of Science and Higher Education of the Russian Federation #0852-2020-0028.

Y. Makarova: None. **A. Alexandrova:** None. **E. Mashkina:** None. **O. Lyangasova:** None.

P07.017.A Retrospective review of genetic testing for inherited bone marrow failure syndromes

Elina Hirvonen[1], *Päivi Kokkonen*[1], *Emma Mårtensson*[1], *Hatice Duzkale*[1], *Joe Jacher*[2], *Alicia Scocchia*[2], *Kim Gall*[2], *Zoe Powis*[2], *Inka Saarinen*[1], *Johanna Sistonen*[1], *Juha Koskenvuo*[1], *Lotta Koskinen*[1], *Tero-Pekka P. Alastalo*[2]

[1]*Blueprint Genetics, a Quest Diagnostics Company, Espoo, Finland,* [2]*Blueprint Genetics Inc, a Quest Diagnostics Company, Seattle, WA, USA.*

Introduction: Inherited bone marrow failure syndromes (IBMFS) are characterized by aplastic anemia, congenital malformations, and increased risk to develop malignancies. Determining the molecular etiology allows for personalized patient management, surveillance, and recurrence risk estimation. Next-generation sequencing (NGS) panel testing is a powerful diagnostic tool. Broad inclusion of genes associated with IBMFS along with copy number variation (CNV) analysis is expected to significantly contribute to diagnostic yield.

Materials and Methods: To determine the diagnostic efficacy of a broad panel test, we reviewed results from IBMFS patients who underwent Bone Marrow Failure Syndrome panel testing. The 135-gene test included sequence variant, CNV, and targeted noncoding variant analysis. CNV analysis included a proprietary method for exon-level CNVs. Variant interpretation followed ACMG guidelines.

Results: Molecular diagnosis was established in 18.6% (82/442) of patients. CNVs contributed to the diagnosis of 17.1% patients; 64.3% of CNVs were intragenic. Diagnostic variants were identified in 34 genes, with 44% identified in a single patient. The most common genes in diagnostic reports included *FANCA* (20.7%) associated with Fanconi anemia and *SBDS* (14.6%) associated with Shwachman-Diamond syndrome. Although variant calling in the *SBDS* gene is

complicated by segmental duplication, sequence variants were identified and confidently mapped to the *SBDS* gene.

Conclusions: This study demonstrates that NGS panel testing with CNV detection contributes to the diagnostic yield among IBMFS patients. Pathogenic variants in *SBDS* were a common finding and testing of this gene should be considered when ordering genetic testing on individuals with, or suspected to have, an IBMFS.

E. Hirvonen: A. Employment (full or part-time); Significant; Blueprint Genetics, a Quest Diagnostics Company. **P. Kokkonen:** A. Employment (full or part-time); Significant; Blueprint Genetics, a Quest Diagnostics Company. **E. Mårtensson:** A. Employment (full or part-time); Significant; Blueprint Genetics, a Quest Diagnostics Company. **H. Duzkale:** A. Employment (full or part-time); Significant; Blueprint Genetics, a Quest Diagnostics Company. **J. Jacher:** A. Employment (full or part-time); Significant; Blueprint Genetics Inc, a Quest Diagnostics Company. **A. Scocchia:** A. Employment (full or part-time); Significant; Blueprint Genetics Inc, a Quest Diagnostics Company. **K. Gall:** A. Employment (full or part-time); Significant; Blueprint Genetics Inc, a Quest Diagnostics Company. **Z. Powis:** A. Employment (full or part-time); Significant; Blueprint Genetics Inc, a Quest Diagnostics Company. **I. Saarinen:** A. Employment (full or part-time); Significant; Blueprint Genetics, a Quest Diagnostics Company. **J. Sistonen:** A. Employment (full or part-time); Significant; Blueprint Genetics, a Quest Diagnostics Company. **J. Koskenvuo:** A. Employment (full or part-time); Significant; Blueprint Genetics, a Quest Diagnostics Company. **L. Koskinen:** A. Employment (full or part-time); Significant; Blueprint Genetics, a Quest Diagnostics Company. **T.P. Alastalo:** A. Employment (full or part-time); Significant; Blueprint Genetics Inc, a Quest Diagnostics Company.

P07.018.B PMA induces both common and distinct genes and pathways across heterogeneous promonocytic cell lines

Duncan A. Cosser, *Kelda C. Perumal, Asavela O. Kama, Nikki L. Gentle*

University of the Witwatersrand, Johannesburg, South Africa.

Introduction: Monocytes and monocyte-derived macrophages exhibit extensive heterogeneity with respect to their phenotypes and functions. This is mirrored in vitro by the promonocytic cell lines commonly used to study the monocyte-to-macrophage transition. We therefore sought to characterise the RNA abundance profiles of three such cell lines, in order to identify genes and pathways core to this process, as well as those uniquely involved in each cell line.

Materials and Methods: RNA sequencing data was obtained across three time points to examine differences in the RNA abundance profiles of HL60, U937, and THP-1 cells treated with and without phorbol 12-myristate 13-acetate (PMA) to induce differentiation to a macrophage state. Differential gene expression analysis across time points was performed using *DESeq2* v1.30.0 and pathway analysis of differentially expressed genes within each cell line was performed using *clusterProfiler* v3.18.1.

Results: We identify genes uniquely expressed within both the differentiated and undifferentiated states of each cell line, as well as a core set of genes that characterise the change in RNA abundance associated with PMA-induced monocyte-to-macrophage differentiation. Pathway analysis of these gene sets reveals the transcription factors and chromatin remodelers involved in this process.

Conclusions: A core RNA abundance signature is associated with PMA-induced differentiation of promonocytic cell lines to a macrophage state. However, differentiation of different cell lines also induces distinct changes in the antiviral profile of each individual cell line.

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P07.019.C Two diseases, one patient. The importance of cytogenetic and FISH in Hematology

Filipa Seixas, *Pedro Sousa, Regina Arantes, Marta Souto, Patricia Ferraz, Osvaldo Moutinho, Manuel Cunha, Rosário Pinto Leite*

Centro Hospitalar Trás os Montes e Alto Douro, Vila Real, Portugal.

Myelodysplastic syndrome is an inefficient cellular process of hematopoiesis. It is a syndrome of cellular proliferation and apoptosis, and it's manifested by a hypercellular bone marrow with cytopenias and affects multiple lineages. This syndrome starts with a genetic mutation in a multipotent hematopoietic progenitor cell.This clinical report is about a female, 82 years old, without relevant personal history, who has normochromic normocytic anemia with hemoglobin of 8.2 g/dL. The endoscopic study with a biopsy of duodenal polyp, showed a follicular lymphoma. To study follicular lymphoma we performed a bone marrow study to rule out the presence of atypical lymphocytes and bone marrow involvement. Through the morphology, cytogenetics and FISH studies, myelodysplastic syndrome was diagnosed. Also, the t(14;18) was executed, which is specif for follicular lymphoma.In conclusion, this patient had one clone of del(11)(q22q35) and another with del(5)(q12q33) confirmed by FISH. The FISH analysis to t(14;18) was negative. Isolated deletion of chromosome 5q (frequency 72%) or del11q (represent 4% of cases) both have a good prognosis.In the literature, these patients have been treated with 5q- isolated syndrome, therefore the patient was proposed to start treatment with Lenalidomide.The presence of these two distinct diseases is an uncommon situation. Cytogenetics plays a significant role in supporting hemato-oncology allowing an accurate diagnosis and consequently targeted therapy

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P07.020.D Immunological profiling of patients with rare short stature, optic nerve atrophy, and Pelger-Huet anomaly (SOPH) syndrome

Leonid Zhozhikov[1], *Ayaan Ivanov*[1], *Roza Ivanova*[1,2], *Aitalina Sukhomyasova*[1,2], *Filipp Vasilev*[1], *Nadezda Maksimova*[1]

[1]*Laboratory of Molecular Medicine and Human Genetics, North-Eastern Federal University (NEFU), Yakutsk, Russian Federation,* [2]*Republican Hospital #1, Yakutsk, Russian Federation.*

Introduction: Pathogenic variant in the neuroblastoma amplified sequence (*NBAS*) gene was described in the Yakut population as a cause of short stature, optic-nerve atrophy, and the Pelger-Huet anomaly of granulocytes (SOPH) syndrome with autosomal recessive inheritance (OMIM #614800). Other mutations in *NBAS* gene have been reported to cause multisystemic disorders with a wide range of phenotypes including recurrent acute liver failure, skeletal dysplasia, eyes pathologies and immunological abnormalities. Although SOPH patients developed a frequent respiratory infection, immunological parameters were not examined.

Materials and Methods: Sampling was carried out from unrelated Yakut patients with SOPH syndrome and healthy individuals. The percent and number of immunocompetent cells were determined by flow cytometry. Immunoglobulin serum levels were determined using ELISA kits. In experiments, we used the equipment of NEFU's Center for Collective Use.

Results: Serum immunoglobulins (IgA, IgM, IgG, IgE) were significantly reduced in SOPH patients in comparison with controls, $CD4^+$ and $CD8^+$ T cells amounts were unremarkable. Patients with SOPH syndrome were characterized by low percentage and number of $CD16^+CD56^+$ NK cells and slightly lower levels of $CD19^+$ B cells.

Conclusions: We suggest that impaired immunological features contribute to recurrent infections in SOPH patients. We would like to emphasize that physicians should pay attention to immunodeficiency in SOPH patients to start appropriate treatment. Functional analysis of the mutational impact on immunocompetent cells is essential to understand the pathophysiology of NBAS disorders. Grant references: This work was supported by the Ministry of Education and Science of Russian Federation (Project No. FSRG-2020-0014).

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P07.021.A Epidemiological of Nosocomial Infection and antimicrobial resistance pattern among different groups of bacteria isolated from some hospitals Jeddah, KSA

Molook Al-Ghamdi[1], *Effat Al-Judaibi*[1], *Mohammed Al-Rashede*[2], *Awatif Al-Judaibi*[1]

[1]*university of jeddah, Jeddah, Saudi Arabia,* [2]*Maternity and Children Hospital Almosadya, Jeddah, Saudi Arabia.*

Nosocomial infections can be described as those that occur within 48 hours of entry to hospital, 3 days of discharge or 30 days of surgery. These infections lead to high morbidity and mortality, prolonged hospital stays, increased use of antibiotics, and increased costs of healing process. Our study was aim to investigate the prevalence, distribution, and antimicrobial susceptibility rates of MDR bacteria in patients with NI from some hospitals in Jeddah city. Bacterial identification and susceptibility testing were performed using modern methods from the microbiology section. The results revealed that based on the percentage distribution of the specimens, the highest number of isolates for *E. coli*, *K. pneumoniae* and *S. aureus*. Was from urine and HVS for *E. coli*, from urine and blood for *K. pneumoniae* and from HVS and wound for *S. aureus*. In order to control and reduce the prevalence of these infection within healthcare settings. we recommend for the importance of surveillance and effective infection control strategies to be implemented among the hospitals and healthcare facilities in Saudi Arabia.

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P07.022.B The impact of persistent infections and human genetic variation on chronic inflammation

Flavia Hodel[1,2], *Olivier Naret*[1,2], *Pedro Marques-Vidal*[3], *Peter Vollenweider*[3], *Jacques Fellay*[1,2,4]

[1]*Global Health Institute, School of Life Sciences, Lausanne, Switzerland,* [2]*Swiss Institute of Bioinformatics, Lausanne, Switzerland,* [3]*Service of Internal Medicine, Department of Medicine, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland,* [4]*Precision Medicine Unit, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland.*

Introduction - Several human pathogens are able to establish chronic, sometimes life-long infections. Even if they are considered latent in the majority of cases, these infections can trigger some degree of local or systemic immune response, resulting in a chronic state of low-grade inflammation. There remains an incomplete understanding of the potential contribution of the interactions between persistent infections and human genetic variation on chronic low-grade inflammation.

Material and methods - We searched for potential associations between seropositivity for a total of 24 persistent pathogens and the plasma levels of the inflammatory biomarker C-reactive protein (CRP), using data collected in the context of the UK Biobank and the CoLaus Study, two large population-based cohorts of European ancestry. We performed linear model analyses for each antigen, including as covariates the demographic and clinical factors associated with CRP as well as polygenic risk scores for CRP (PRS-CRP), calculated separately on each cohort.

Results: We observed increased CRP levels with each increase in PRS-CRP quartiles. We also found evidence for an involvement of *Chlamydia trachomatis* ($p = 0.004$), *Helicobacter pylori* ($p = 0.018$), Cytomegalovirus ($p = 0.025$) and Kaposi's sarcoma-associated herpesvirus ($p = 0.041$) infection in the determination of increased plasma levels of CRP.

Conclusions - The results of this study improve our understanding of the relationship between chronic, subclinical infections and human health. These could lead to better disease prediction models and to the identification of potential novel targets for diagnostic or therapeutic development. Grant reference: #175603 (Swiss National Science Foundation)

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P07.023.C Identification of copy number variants relevant to primary immunodeficiency from exome sequencing data

Rensheng Wan[1], *Maximilian Schieck*[1], *Winfried Hofmann*[1], *Philipp H. B. Knopf*[1], *Michele Proietti*[2,1], *Andres Caballero Garcia de Oteyza*[2], *Thomas Illig*[1], *Bodo Grimbacher*[2,1], *Doris Steinemann*[1]

[1]*Hannover Medical School, Hannover, Germany,* [2]*University of Freiburg, Freiburg, Germany.*

Introduction: Primary antibody deficiencies (PADs) comprise a group of heterogeneous disorders with defects in B cell development or function. A genetic cause is often suspected, however pathogenic sequence variants are typically found in less than 30% of PAD patients. This study focuses on copy number variants (CNVs) and whether the diagnostic yield might be improved by routine CNV detection.

Materials and Methods: Potential CNVs were called with the ClinCNV algorithm from whole exome sequencing (WES) data from 200 PAD patients. Stringent filtering based on 430 loci related to PID, internal quality control parameters, and the database of genomic variants. Independent validation of CNVs was done by SNP-array.

Results: A total of 24 CNVs in 23 patients were validated. This suggests presence of potentially clinically meaningful CNVs in 11.5% of this PAD cohort. Two patients had heterozygous microdeletions in chromosome *22q11.2*. Abnormal lymphocyte proliferation, hypogammaglobulinemia, and autoimmune hemolytic anemia observed in both patients seem accountable to *22q11.2* microdeletion. Two patients had homozygous deletions of

ICOS exons 2 and 3, representing a known founder variant. Consistent with ICOS deficiency, patients had hypogammaglobulinemia, splenomegaly, and thrombocytopenia. Another 19 patients had CNVs in a broad range of PID-related genes, e.g. *LRBA, TNFAIP3, PSTPIP1, MEFV, FANCA, TYK2, and IKBKG*.

Conclusions: CNV analysis from WES data has potential to increase the diagnostic yield in the PAD cohort substantially. Functional analysis of newly identified CNVs needs to follow. **Grants:** Deutsche Forschungsgemeinschaft (DFG) under Germany's Excellence Strategy - EXC 2155 - Projektnummer 390874280.R. Wan holds a DAAD scholarship.

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P07.024.D PADI4 and PADI2 enhance collagen-initiated inflammatory responses

Akari Suzuki, *Takumi Shibuya, Kazuhiko Yamamoto*

RIKEN, Yokohama, Japan.

Previously, peptidylarginine deiminase type 4 **(**PADI4) was identified as a susceptibility gene for Rheumatoid arthritis (RA) by genome-wide association studies. Peptidyl citrulline is a target antigen of anti-citrullinated peptide antibodies (ACPAs), and only PADs (translated protein from PADI genes) can provide peptidyl citrulline via modification of protein substrates. Also the distribution of PADI4 and PADI2 has overlap in immune cells. The aim of this study is to investigate the relationship between PADI4 gene and PADI2 gene in the progression of RA. To clarify the physiological function of PADI4 and PADI2 in RA, we used collagen-induced arthritis (CIA), known as a RA model mouse. We examined that localization of PAD4 and PAD2 protein was indicated by immunohistochemistry in CIA mice. We also measured expression of Padi genes and various inflammatory cytokines in immune cells by real-time TaqMan assay and ELISA, respectively. We generated PADI4−/− and PADI2−/− mice and performed experimental arthritis. We demonstrated that the clinical disease score was significantly decreased in PADI4−/− mice and PADI4 expression was induced by CII immunization. In PADI4−/− mice sera, serum anti-type II collagen (CII) IgM, IgG, and inflammatory cytokine levels were also significantly decreased compared with those in wild-type mice sera. Interestingly, PADI2 expression was compensationally induced in CD11b+ cells of PADI4-/- mice. We examined that the clinical disease score of CIA mimce and expression levels of Padi genes in PADI2−/− CIA mice. It appears that PADI4 and PADI2 enhance collagen-initiated inflammatory responses. We are currently working on a double knockout mouse.

A. Suzuki: None. **T. Shibuya:** None. **K. Yamamoto:** None.

P07.025.A Genotyping of Russian patients with RA using the targeted NGS panel

Ekaterina A. Vetchinkina[1], *Dmitriy S. Mikhaylenko*[1,2], *Ekaterina B. Kuznetsova*[1,2], *Ekaterina A. Alekseeva*[1], *Irina V. Bure*[1], *Marina V. Nemtsova*[1,2]

[1]*First Moscow State Medical University named I.M. Sechenov, Moscow, Russian Federation,* [2]*Research Center for Medical Genetic, Moscow, Russian Federation.*

Introduction: Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by synovial inflammation and destruction of cartilage and bone. RA is considered as a multifactorial disease triggered by a genetic predisposition and environmental factors. Unfavorable alleles of various genes have a relatively small influence on the disease risk when they appear individually, but in combination, they predispose an individual to RA development.

Materials and methods: Genotyping of 85 SNPs and 6 complete genes was performed using NGS on a target panel (IAD177464_185) in 125 patients with RA. The allele frequencies from the GnomAD base for Caucasian were used as control. Statistical analysis was performed by STATISTICA version 13.5.0. The result values were corrected using the Benjamini-Hochberg method.

Results: According to our data, the alleles HLA-DRB1*04, HLA-DRB1*01, HLA-B27, PTPN22 (rs2476601), TNF (rs1800629), TPMT (rs2842934), IL4 (rs2243250) and genotypes HLA-DRB1*04*04, HLA-DRB1*01*16, PTPN22 (rs2476601), TPMT (rs2842934) were significantly associated with the development of the disease in patients. We investigated of associations with clinical criteria (DAS28-CRP, HAQ-DI, CDAI) and biochemical factors (ACPA formation, RF, CRP). We have shown that the PADI4 genotypes (rs11203367, rs2240340, rs11203366, rs874881) are significantly associated with the baseline levels of DAS28-CRP, HAQ-DI, CDAI, genotypes IL23R (rs7530511), TNFRSF1A (rs748004, rs2228144) with the level of ACPA, the genotypes DHODH (rs3213422), MTHFR (rs180113) with the concentration of CRP, and the genotypes IL2RA (rs2104286), IRAK3 (rs11541076) and IL4R (rs1801275) with the level of RF.

Conclusions: Application of targeted NGS panel contributes to expanded genotyping to identify risk groups among Russian patients with RA.

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P07.026.B A very rare case of immunodeficiency with autoinflammation

Mihaela T. Bataneant[1], *Adela Chirita-Emandi*[2], *Estera Boeriu*[2], *Mihaela Baica*[2], *Andreea Beloia*[2], *Patricia Urtila*[2]

[1]*University of Medicine and Pharmacy "Victor Babes", Romania, Romania,* [2]*University of Medicine and Pharmacy "Victor Babes", Timisoara, Romania.*

Introduction: Infection with recurrent fever is very suggestive for primary immunodeficiency, but immunodeficiency associated with autoinflammation is very rare recognized. Case presentation. A 1 year old female, from non-consanguineous parents and no significant family history was admitted for suspicion of immunodeficiency. Since 3 months of age, after the vaccination, she presented at each 2-3 weeks fever, vomiting and diarrhea for 5-10 days. Clinical exam revealed a Down sd. like face, psychomotor delay, microcephaly. Laboratory tests showed a microcytic hypochromic anemia with

normal ferritin confirmed as sideroblastic anemia by bone marrow aspiration, variable leucocyte(2200 - 8000/µL), neutrophil(630-4500/µL) and lymphocyte count(1180-3500/µL), negative coprocultures and coproantigenes, hypogammaglobulinemia(1,7%), a C reactive protein >100 mg/L. Down sd., celiac disease, hypothyroidism, cystic fibrosis, Schwachmann-Diamond sd, IBD were excluded. Immunological explorations showed low IgA, IgG and IgM, poor response to vaccination, B lymphopenia and low switched memory B cells. Taking in account the early onset, hypogammaglobulinemia and periodic fever with the similar picture we performed WES that revealed double heterozigous c.608+1G > T/c.1246A > G missense mutation in TRNT1 gene causing SIFD (sideroblastic anemia with B cell immunodeficiency, periodic fever and developmental delay). She started immunoglobulin substitution. Due to severe prognosis and because 2 patients were successfully bone marrow transplantated we take in discussion to perform BMT in this case.

Conclusion: SIFD is a very rare immunodeficiency, 33 patients being reported until present. It must be suspected in each case of periodic fever with the similar picture associated with hypogammaglobulinemia and developmental delay, genetic exploration being crucial.

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P07.027.C Biological miR-146a-TRAF6 axis is associated with lupus flares and renal fibrosis progression

Olga Martinez-Arroyo[1], *Ana Ortega*[1], *Javier Perez-Hernandez*[2], *Ana Flores*[1], *Josep Redon*[3], *Maria J. Forner*[3], *Raquel Cortes*[1]

[1]*Biomedical Research Institute Clinic Hospital - INCLIVA, Valencia, Spain,* [2]*INSERM, U1016, I, Cochin Institute, Paris, France,* [3]*Universitary Clinic Hospital, Valencia, Spain.*

Introduction: Urinary exosomes, especially microRNAs (miRNAs) packaged within, are ideal sources of renal damage markers. We investigated the association between exosomal miR-146a, (anti-inflammatory regulator) and disease activity, proteinuria and systemic lupus erythematosus (SLE) flares over a 36-month follow-up period.

Methods: We isolated urinary exosomes from 41 SLE patients, 27 with lupus nephritis (LN) and 20 healthy controls, and exosomal miR-146a, quantified by the real-time quantitative polymerase chain reaction (RT-qPCR), was correlated with histological features in 13 renal biopsies. We also analysed the association between the exosomal miR-146a and TNF receptor associated factor 6 (TRAF6 axis).

Results: Exosomal miR-146a showed an inverse association with circulating C3 and C4 complement components, proteinuria, and with histological features such as chronicity index. This marker was able to identify LN with an AUC of 0.82 ($p = 0.001$). Basal exosomal miR-146a was associated with disease activity and proteinuria changes and was an independent marker of 36-month follow-up flares (OR 7.08, $p = 0.02$). Pathway analysis identified IRAK1 and TRAF6 as miR-146a target genes. Finally, in vitro experiments suggested that miR-146a exerts a protective effect through negative regulation of inflammation by suppressing IRAK1 and TRAF6.

Conclusions: Urinary exosomal miR-146a levels are correlated with lupus activity, proteinuria and histological features, discriminating patients with LN and being a good baseline marker of SLE flares. We have identified a relevant biological miR-146a-TRAF6 axis association in LN renal fibrosis progression. Funding from Carlos III Health Institute (PI18/01405, CD18/00166) and with ERDF.

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P07.028.D Association toll-like receptor TLR2 and TLR9 gene polymorphism and carriage of *Staphylococcus aureus* in children with recurrent respiratory infections

Inna Pokudina, *Elizaveta Peshehodko*

Southern Federal University, Rostov-on-Don, Russian Federation.

Introduction: Recurrent respiratory infections (RRI) in children represent a social issue. RRI are mainly caused by viruses, however, their course is often complicated by *Staphylococcus aureus* infections. Toll-like receptors play an important role in the activation of the immune system by regulating the production of antimicrobial peptides and inflammatory cytokines. This study aimed to explore the association between the gene polymorphisms of *TLR2, TLR9* and the nasopharyngeal carriage of *Staphylococcus aureus* in children with RRI.

Material and methods: The polymorphisms *TLR2 (Arg753Gln*) and *TLR9 (T-1237C*) were genotyped in 48 children with RRI (≥4 episodes in the year) 2-10 years old. 20 children were with known *S. aureus* nasopharyngeal carrier status and 18 noncarriers (control group). Genomic DNA was extracted from blood of participants, genotyping was performed using RT-PCR.

Results: No differences were found between carriers and noncarriers regarding the allelic ($\chi2=0{,}84$; $p = 0{,}358$) and genotype ($\chi2=0{,}926$; $p = 0{,}63$) distribution of the *TLR2*. For the polymorphism of *TLR9*, statistically significant differences were found in the distribution of allele ($\chi2 = 8.161$; $p = 0.005$) and genotypes ($\chi2 = 7.538$; $p = 0.024$) frequencies. Carriage of dominant homozygous *T/T TLR9* trait increases the likelihood to protect against *S. aureus* (OR = 0,11; 95 % CI 0,02-0,63), heterozygous T/C increases the risk (OR = 5,85; 95 % CI 1,03-32,79).

Conclusion: Our study indicated the potential role the polymorphisms *TLR9 (T-1237C)* as the genetic marker of predisposition to *Staphylococcus aureus* carriage. This study was funded by the Ministry of Science and Higher Education of the Russian Federation #0852-2020-0028.

I. Pokudina: None. **E. Peshehodko:** None.

P07.029.A Molecular spectrum of Von Willebrand Disease Type 3

Esra Isik[1], *Durdugul Ayyildiz Emecen*[1], *Kaan Kavakli*[1], *Fahri Sahin*[1], *Enise Avci Durmusalioglu*[1], *Canan Albayrak*[2], *Melike Evim*[3], *Ekrem Unal*[4], *Fatma B. Belen*[5], *Neslihan Karakurt*[6], *Ozgur Cogulu*[1], *Ferda Ozkinay*[1], *Tahir Atik*[1]

[1]*Ege University Faculty of Medicine, Izmir, Turkey,* [2]*Ondokuz Mayıs University Faculty of Medicine, Samsun, Turkey,* [3]*Uludağ University Faculty of Medicine, Bursa, Turkey,* [4]*Erciyes University Faculty of Medicine, Kayseri, Turkey,* [5]*Başkent University Faculty of Medicine, Ankara, Turkey,* [6]*Sancaktepe Prof. Dr. İlhan Varank Training and Research Hospital, İstanbul, Turkey.*

Aim: Von Willebrand disease (VWD) is the most common inherited bleeding disorder caused by patogenic variants in *VWF* gene. The prevalence of VWD is reported between 0.1-1%. The disease is classified into 3 subtypes due to the qualitative or quantitative disorder of von Willebrand factor. Pathogenic variants can be

detected via a *VWF* sequence analysis, in 65% of Type 1 and 80% of Type 3 patients. In this study, molecular spectrum of 29 patients from 28 different families followed up with VWD type 3 was evaluated.

Material and Method: 29 patients from 28 different families with a pre-diagnosis of VWD were included in the study. The clinical features and laboratory findings of patients were obtained from their hospital records. The *VWF* gene was sequenced by the next generation sequence analysis method. The detected variants were investigated in HGMD (Human Genome Mutation Database) and EAHAD-CFDB (EAHAD Coagulation Factor Variant Databases). ACMG criteria were used to evaluate the pathogenicity of variants.

Results: In our study, 17 of the patients were female. In *VWF* gene analysis, 21 different variants associated with the VWD type 3 were identified and nine of them were frameshift, five missense, five nonsense and two splice site mutation. Seven (c.3533delC, c.1898G>A, c.6589delG, c.1486G>T, c.6239delA, c.480delG, c.2733dupT) of the variants had not been previously reported in the literature.

Conclusion: By defining seven novel mutations, this study enriched to the molecular spectrum of VWD type 3, while also providing a further insight for genetic counselling.

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P07.030.B New "ural" variants of *BTK*-gene in Russian patients with agammaglobulinemia

Svetlana Deryabina[1,2,3], *Elena Vlasova*[4], *Irina Tusankina*[1,2]

[1]*The Ural Institute of Immunology and physiology, Ekaterinburg, Russian Federation,* [2]*Federal State Autonomous Educational Institution of Higher Education «Ural Federal University named after the first President of Russia B.N.Yeltsin, Ekaterinburg, Russian Federation,* [3]*Medical Center "Health Care of Mother and Child", Ekaterinburg, Russian Federation,* [4]*Sverdlovsk Regional Children Clinical Hospital, Ekaterinburg, Russian Federation.*

Background: X-linked agammaglobulinemia is a primary disorder of humoral immunity, the main symptom of which is a deficiency of B cells. The BTK gene associated with pathology has more than 500 mutations, including single base pair substitutions, splicing defects, and small deletions and insertions. Since 2014 seven patients with a deficiency of the B-cell were received molecular genetic confirmation for this congenital error of immunity. One child was diagnosed in first year of life, two children were examined at 2 years old, another 4 at the age of 5 years. Periodically respiratory diseases occurred in childhood of this babies did not alert district doctors, only acute clinical manifestations of the disease they were sent a case to immunologist.

Results: We identified 7 causative genetic variants by the targeted sequencing of the *BTK*-gene. Four mutations previously reported in the *BTK* database and three variants out of 7 detected are new*. Table 1. Results of the molecular genetic study of patients with XLA

No. patient	New variant of *BTK*-gene (GRCh38, NM_000061)
1	c.1051_1052insA (p.Gln297AlafsTer26)*
2	c.928_929insA (p.Ser310LysfsTer13)*
3	c.64_76del13(delCCTCTAAACTTCA), (p.P22fsTer28)*

Conclusion. Seven patients has a molecular verification for the diagnosis X-linked agammaglobulinemia. Four families (4 mothers, 1 aunt, 2 siblings) were issued a conclusion on the family variant of the pathology inheritance. This information allow parents to carry out prenatal diagnosis of X-linked disease in the fetus during the next pregnancy, and siblings have have important information for planning their families in the future.

S. Deryabina: None. **E. Vlasova:** None. **I. Tusankina:** None.

P08 Intellectual Disability

P08.001.C Increasing the diagnostic yield of copy number variation using an exonic aCGH

Neus Baena, *Elisabeth Gabau, Núria Capdevila, Juan Pablo Trujillo, Carmen Mata, Anna Ruiz, Nino Spataro, Miriam Guitart*

Corporació Sanitària Parc Taulí, SABADELL, Spain.

Introduction: The increase in resolution and coverage of aCGH is of particular importance for genes implicated in neurodevelopmental disorders that are subject to copy number variation (CNV). The aim is to evaluate the diagnostic yield achieved using an exonic aCGH platform and to determine the rate of missed cases between two different platforms.

Material and Methods: An 60k aCGH (Agilent Technologies) was first used as a first tier test for neurodevelopmental disorders from 2013 to 2019. In 2020 we have incorporated a high-resolution exon targeted aCGH of 180k (Oxford Gene Technology, OGT).

Results: Using the OGT technology aCGH the diagnostic yield increased from 10.6% to 15%. A 3.4% of cases would not have been diagnosed using the low density platform. It corresponds to 14 cases (11 deletions and 3 duplication). The size of CNV ranges between 2.2 kb to 243 kb. All these case were confirmed using customized MLPA. Among those cases, some are of particular interest: exon 45 deletion in *DMD* gene, exon 2 deletion of *TMLHE* and downstream CN5-ECR1-CN9 enhancers deletion of *SHOX*.

Conclusions: Increasing aCGH resolution to single exons led to detection of small CNVs in known disease genes, some intragenic CNVs can easily be missed using a lower resolution array. Our findings highlight the importance of high-resolution aCGH and the critical analysis of aCGH data surrounding genes that are involved by genomic variation.

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P08.002.D Refining the phenotype and expanding the genotype of Xia-Gibbs Syndrome (OMIM #615829)

Ana Teresa Serrano Antón[1,2], *María José Sánchez Soler*[1,2], *Mary Ballesta Martínez*[1,2], *Vanesa López González*[1,2], *Lidia Rodríguez Peña*[1,2], *Encarna Guillén Navarro*[1,2]

[1]*Sección de Genética Médica. Hospital Clínico Universitario Virgen de la Arrixaca, IMIB-Arrixaca, Murcia, Spain,* [2]*Grupo Clínico Vinculado al Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Instituto de Salud Carlos III (ISCIII), Madrid, Spain.*

Introduction: Xia-Gibbs syndrome (XGS, OMIM#615829) results from de novo truncating variants within the AHDC1 gene. About 100 patients worldwide have been identified. The spectrum of manifestations comprises hypotonia, developmental delay, intellectual impairment, brain structural anomalies, sleep difficulties, growth/feeding issues and dysmorphic facial features. The phenotype is still expanding.

Material and Methods: Retrospective descriptive study of patients with XGS diagnosis at clinical genetics service of Spanish tertiary-level hospital.

Results: We describe four males, ages between 7 and 24 years, born to healthy non-consanguineous parents. During pregnancy, structural ultrasounds were normal. All presented with hypotonia, developmental delay (2/4 absent expressive speech) and variable intellectual disability. All show stereotypic demeanor, three with behavior disorder. Brain structural anomalies were demonstrated in everyone by MRI: cortical dysplasia, asymmetric ventriculomegaly, hydrocephaly and fossa posterior anomalies. Whole patients with strabismus, two refractive error added. Cryptorchidism in 3/4. Two exhibit patellar subluxations. One manifested feeding difficulties with growth failure at infancy and nocturnal snoring. Facial features, nonspecific, in all: depressed nasal bridge, thin upper lip and horizontal eyebrows. Whole exome sequencing (WES) revealed de novo heterozygous truncating variants at AHDC1 gene: two nonsense (c.2260C>T and c.2062C>T) and two frameshit (c.2641_2644dupGCCC and c.2565delT), none of them previously reported.

Conclusions: We report on four new cases of XGS. Hypotonia, intellectual impairment, stereotypic demeanor, brain structural anomalies and strabismus compose their phenotype. Sleep/feeding difficulties appear in only one. Moreover, dysmorphism do not demonstrate a recognizable pattern. This emphasize WES to identify new cases and to provide additional clinical and molecular data.

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P08.003.A Clinical and molecular characteristics of 44 Tunisian patients with Angelman Syndrome

Hana Fredj[1], *Asma Azaza*[2], *Lilia Kraoua*[1], *Ahlem Achour*[1], *Hedia Klaa*[3], *Ichraf Kraoua*[3], *Melek Trigui*[1], *Rym Meddeb*[1], *Ines Ouertani*[1], *Neila Belghith*[1], *Faouzi Maazoul*[1], *Ridha Mrad*[1], *Madiha Trabelsi*[1]

[1]*Department of Congenital and Hereditary Diseases, Charles Nicolle Hospital, Tunis, Tunisia,* [2]*Faculty of Medicine of Sfax, University of Sfax, Sfax, Tunisia,* [3]*Department of Child and Adolescent Neurology, Institute Of Neurology Mongi Ben Hmida, Tunis, Tunisia.*

Introduction: Angelman syndrome (AS) (NM_105830) is a neurodevelopmental disorder characterized by developmental delay, intellectual disability, severe speech impairment, movement or balance disorder, seizures and characteristic abnormal behavior. AS is caused by the loss of expression of the maternal copy of the *UBE3A* gene in 15q11.2-q13 imprinted region. Many genetic mechanisms are involved, of which the most frequent was the deletion of the maternally inherited 15q11.2-q13 region.

Materials and Methods: Clinical and genetic analyses of a cohort of 44 patients, referred to our Department of Congenital and Hereditary Diseases from 2004 to 2020. MS-PCR was performed for diagnostic confirmation while FISH, microsatellites study and *UBE3A* gene sequencing were realized to genetic mechanism determination.

Results: We noted one familial form with 6/44 affected cases and 38/44 sporadic ones. The sex ratio was 1,44. The average age at the first presentation was 4 years and at the diagnosis confirmation was 4.5 years. All patients had developmental delay, severe speech impairment and characteristic behavior. Seizures and ataxia were noted in 81.8 and 72.7% of patients, respectively. The physical examination revealed hypopigmentation (72.7%), an evocative facial dysmorphia. 70.4%, microcephaly (63.6%) and strabismus (68%). The follow-up was possible for only 59% of patients. The genetic mechanisms were 15q11-q13 microdeletion (30/44), *UBE3A* mutations (8/44) and maternal DUP15 (3/44).

Conclusion: We studied the largest cohort of AS in Tunisia. A better knowledge of AS clinical features will allow an earlier diagnosis and an adapted care.The determination of the genetic mechanism refines the genetic counselling.

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P08.004.B Haploinsuffiency of *ARFGEF1* is associated with developmental delay, intellectual disability and epilepsy with variable expressivity

Quentin Thomas[1,2,3], *Thierry Gauthier*[4], *Dana Marafi*[5,6], *Thomas Bernard*[7,8], *Marjolaine Willems*[9], *Sébastien Moutton*[1,3], *Bertrand Isidor*[7,8], *Benjamin Cogné*[8,7], *Solène Conrad*[7,8], *Romano Tenconi*[10], *Maria Iascone*[10], *Arthur Sorlin*[1,3,11], *Alice Masurel*[1,3], *Tabib Dabir*[12], *Adam Jackson*[13], *Siddharth Banka*[13,14], *Julian Delanne*[1], *James R. Lupski*[5,15,16], *Nebal W. Saadi*[17,18], *Fowzan S. Alkuraya*[19,20], *Fatema Al Zahrani*[19], *Pankaj B. Agrawal*[21,22], *Eleina England*[23], *Jill A. Madden*[24], *Jennifer E. Posey*[5], *Lydie Burglen*[25,26], *Diana Rodriguez*[27], *Martin Chevarin*[3], *Sylvie Nguyen*[28], *Frédéric Tran Mau-Them*[3,28], *Yannis Duffourd*[3], *Philippine Garret*[3], *Ange-Line Bruel*[3], *Patrick Callier*[3,28], *Nathalie Marle*[3], *Anne-Sophie Denomme-Pichon*[3,28], *Laurence Duplomb*[3,28], *Christophe Philippe*[28,3], *Christel Thauvin-Robinet*[1,1,29], *Jérôme Govin*[4], *Laurence Faivre*[1,3,30], *Antonio Vitobello*[3,28]

[1]*Genetics Center, FHU-TRANSLAD, Dijon Bourgogne University Hospital, Dijon, France,* [2]*Department of Neurology, Dijon Bourgogne University Hospital, Dijon, France,* [3]*Inserm UMR1231 team GAD, University of Burgundy and Franche-Comté, Dijon, France,* [4]*Institute for Advanced Biology, Centre de Recherche UGA, INSERM U1209CNRS UMR5309, Site Santé, La Tronche, France,* [5]*Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA,* [6]*Department of Pediatrics, Faculty of Medicine, Kuwait University, Safat, Kuwait,* [7]*Service de génétique médicale, CHU Nantes, Nantes, France,* [8]*Université de Nantes, CNRS, INSERM, l'institut du thorax, Nantes, France,* [9]*Unité INSERM U 1051, Département de Génétique Médicale, CHRU de Montpellier, Montpellier, France,* [10]*University of Padova, Laboratorio Genetica Medica Bergamo, Bergamo, Italy,* [11]*Functional Unit of Innovative Diagnosis for Rare Diseases, Dijon Bourgogne University Hospital, Dijon, France,* [12]*Medical genetics Department, Belfast City Hospital, UK BT9 7AB, Belfast, Ireland,* [13]*Division of Evolution & Genomic Sciences, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom,* [14]*Manchester Centre for Genomic Medicine, St Mary's Hospital, Manchester University NHS Foundation Trust, Health Innovation Manchester, Manchester, United Kingdom,* [15]*Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA,* [16]*Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX, USA,* [17]*College of Medicine, University of Baghdad, Baghdad, Iraq,* [18]*Children Welfare Teaching Hospital, Baghdad, Iraq,* [19]*Department of Genetics, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia,* [20]*Department of Anatomy and Cell Biology, College of Medicine, Alfaisal University, Riyadh, Saudi Arabia,* [21]*Divisions of Newborn Medicine and Genetics & Genomics, Manton Center for Orphan Disease Research, Boston, MA, USA,* [22]*Department of Pediatrics, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA,* [23]*Center for Mendelian Genomics, Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, MA, USA,* [24]*The Manton Center for Orphan Disease Research, Division of Genetics & Genomics; Boston Children's Hospital, Boston, MA, USA,* [25]*Centre de Référence des Malformations et Maladies Congénitales du Cervelet, et Département de Génétique, AP-HP.Sorbonne Université, Hôpital Trousseau, Paris, France,* [26]*Developmental Brain*

Disorders Laboratory, Imagine Institute, INSERM UMR 1163, Paris, France, [27]APHP, Service de Neuropédiatrie, Hôpital Armand Trousseau, UPMC Université, Inserm U676, Paris, France, [28]Functional Unit of Innovative Diagnosis for Rare Diseases, Dijon Bourgogne University Hospital, Dijon, France, [29]Centre de référence Déficiences Intellectuelles de Causes Rares, Dijon Bourgogne University Hospital, Dijon, France, [30]Centre de Référence Anomalies du Développement et Syndromes Malformatifs, Dijon Bourgogne University Hospital, Dijon, France.

Introduction: ADP Ribosylation Factor Guanine nucleotide Exchange Factors (ARFGEFs) are a family of proteins implicated in cellular trafficking between the Golgi apparatus and the plasma membrane through vesicle formation. Amongst them, ARFGEF1/BIG1, involved in axon elongation, neurite development and polarization processes, has been previously suggested as a candidate gene for rolandic epilepsy, familial febrile convulsions and epileptic encephalopathy. However, its implication in human disease has not been confirmed so far.

Methods and Results: Through international data sharing, we identified 13 individuals presenting with neurodevelopmental disorders (NDDs) and carrying heterozygous likely damaging variants in *ARFGEF1*, identified as a candidate research gene after negative clinical exome analyses. These individuals displayed congruent clinical features of motor developmental delay (12/13), speech delay (12/13), behavioral disorders (12/13), intellectual disability (10/13), brain neuroanatomical findings (7/13), and epileptic disorders (6/13), although phenotypes were variable, even within families. While almost half of the cohort carried *de novo* variants, at least 40% of variants were inherited from mildly affected parents, clinically reevaluated by reverse phenotyping. Our functional assays show that the mechanism underlining *ARFGEF1*-related conditions is consistent with haploinsufficiency.

Conclusions: Overall, these results show that pathogenic variants in *ARFGEF1* are responsible for sporadic and familial cases of NDDs with variable expressivity. In this respect, some individuals carried rare variants found in the Genome Aggregation Database - gnomAD, indicating that phenotypes may be very mild. Eventually, *ARFGEF1* should be routinely screened in unsolved cohorts of individuals presenting with neurodevelopmental disorders with or without epilepsy.

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P08.005.C Inherited *ARID1B* variants: evidence of non-pathogenicity or variable expression?

P.J. van der Sluijs[1], *M. Alders*[2], *A Dingemans*[3], *E Gerkes*[4], *B.W. van Bon*[5], *J.C. Dempsey*[6], *D Doherty*[6,7], *I Miller*[8], *J.A. Rosenfeld*[9,10], *S Moortgat*[11], *K Parbhoo*[12], *M Pastore*[12], *D Regier*[8], *B Schmalz*[12], *T Smol*[13], *K.E. Stuurman*[14], *B.B.A. de Vries*[3], *S.E. Hickey*[12,15], *I Maystadt**[11], *G.W.E. Santen**[1]

[1]*Department of Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands,* [2]*Amsterdam UMC, University of Amsterdam, Department of Clinical Genetics, Amsterdam, Netherlands,* [3]*Department of Human Genetics, Donders Institute for Brain, Cognition and Behaviour, Radboud university medical center, Nijmegen, Netherlands,* [4]*Department of Genetics, University of Groningen, University Medical Center Groningen, Groningen, Netherlands,* [5]*Department of Human Genetics, Radboud University Medical Center, Nijmegen, Netherlands,* [6]*Department of Pediatrics, University of Washington, Seattle, WA, USA,* [7]*Center for Integrative Brain Research, Seattle Children's Research Institute, Seattle, WA, USA,* [8]*Genetics and Metabolism, Children's National Hospital, Washington, WA, USA,* [9]*Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA,* [10]*Baylor Genetics Laboratories, Houston, TX, USA,* [11]*Centre de Génétique Humaine, Institut de Pathologie et de Génétique, Gosselies, Belgium,* [12]*Division of Genetic & Genomic Medicine, Nationwide Children's Hospital, Columbus, OH, USA,* [13]*Service de génétique clinique Guy Fontaine, CHRU de Lille-Hôpital Jeanne de Flandre, Lille, France,* [14]*Erasmus MC, University Medical Center Rotterdam, Department of Clinical Genetics, Rotterdam, Netherlands,* [15]*Department of Pediatrics, The Ohio State University College of Medicine, Columbus, OH, USA.*

ARID1B is one of the most frequently mutated genes in intellectual disability (~1%). Most variants in *ARID1B* are readily classified, since pathogenic variants are usually *de novo* and truncating. Recently we have shown that the *ARID1B* phenotype can include normal IQ values, suggesting that a pathogenic variant may be inherited from a very mildy affected parent. We also found that some regions seem to be devoid of *ARID1B* mutations suggesting that truncating variants here may not lead to *ARID1B* haploinsufficiency. Thus, for some truncating variants, in particular those which are inherited, it may be difficult to make the distinction between non-pathogenic variants or pathogenic variants with variable expression. We collected patients with potentially truncating variants which could not be readily classified because of inheritance or the location of the variants, and performed DNA methylation analysis. In total, 12 patients were included. With clinical and DNA methylation studies we were able to confidently classify most variants. We thus identified 5 families with transmitted pathogenic variants confirming highly variable expression. We also provide further evidence of an alternative start-site, which is located between cDNA position 363-521, and formulate guidelines for the interpretation of *ARID1B* variants. *shared last author

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P08.006.D Clinical phenotype associated with *ARID2* pathogenic variants: a report of twelve new cases and literature review

Clara Houdayer[1], *Alban Ziegler*[1], *Céline Bris*[1], *Alice Goldenberg*[2], *Antoine Bonnevalle*[2], *Mélanie Fradin*[3], *Christèle Dubourg*[3], *Marie Vincent*[4], *Benjamin Cogné*[4], *Sandra Whalen*[5], *Boris Keren*[5], *Christel Tauvin*[6], *Arthur Sorlin*[6], *Ange-Line Bruel*[6], *David Geneviève*[7], *Rebecca Procopio*[8], *Karen Gripp*[8], *Jennifer Schleit*[9], *Brice Mendelsohn*[9], *Olivier Patat*[10], *Marine Tessarech*[1], *Agnès Guichet*[11], *Dominique Bonneau*[11], *Bertrand Isidor*[4], *Estelle Colin*[1]

[1]Department of Biochemistry and Genetics, Angers University Hospital, Angers, France, [2]Department of Genetics and Reference Centre for Developmental Disorders, Rouen University Hospital, Rouen, France, [3]Department of Genetics, Rennes University Hospital, Rennes, France, [4]Department of Genetics, Nantes University Hospital, Nantes, France, [5]Department of Genetics, APHP, GH Pitié-Salpêtrière, Paris, France, [6]Department of Genetics, Dijon University Hospital, Dijon, France, [7]Department of Genetics, Montpellier University Hospital, Montpellier, France, [8]Division of Medical Genetics, AIdu Pont Hospital for Children, Wilmington, DE, USA, [9]Division of Medical Genetics, University of California, San Francisco, CA, USA, [10]Department of Genetics, Toulouse University Hospital, Toulouse, France, [11]Department of Genetics, Angers University Hospital, Angers, France.

ARID2 (AT-rich interaction domain 2) is a newly described disease-causing gene encoding a protein belonging to the SWI/SNF complex, an ATP-dependent chromatin remodeling complex which regulates DNA accessibility at the nucleosome and facilitates DNA transcription, replication and repair. *ARID2* acts as a tumor suppressor and has also been involved in intellectual disabilities including "SWI/SNF-related intellectual disability disorders" (SSRIDDs). Furthermore, it has been shown very recently that *ARID2* haploinsufficiency is associated with enhanced RAS-MAPK activity. To date, twenty-two individuals have been reported with intragenic *ARID2* mutations or deletions at chromosome 12q12-13.11, where *ARID2* is located. These individuals share common features including intellectual disability, hypotonia, behavioral disorders, short stature, and dysmorphic features that may overlap with those of RASopathies. In order to further delineate the *ARID2* phenotypic spectrum, we report a cohort of twelve unrelated individuals harboring *ARID2* deletion or pathogenic variants and we compare their features with those previously described. The clinical characteristics of individuals from this series seem to be more moderate, in particular, some individuals had mild or even absent intellectual disability and they had more moderate growth abnormalities. Behavioral problems, anxiety and attention-deficit hyperactivity disorder appear to be common features of this condition.

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P08.007.A Investigating the "dark" genome reveals the first family with Partington syndrome in Cyprus

Constantia Aristidou[1,2], *Athina Theodosiou*[3,2], *Elena Spanou-Aristidou*[1], *Violetta Christophidou-Anastasiadou*[4,1,2], *Carolina Sismani*[3,2], *George A. Tanteles*[1,2]

[1]Clinical Genetics Clinic, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus, [2]The Cyprus School of Molecular Medicine, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus, [3]Department of Cytogenetics and Genomics, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus, [4]Department of Clinical Genetics, Archbishop Makarios III Medical Centre, Nicosia, Cyprus.

We present a large four-generation family having multiple affected male individuals with a variable intellectual disability (ID), hand dystonia and epilepsy phenotype, segregating in an X-linked manner. Extensive genetic testing including whole-exome and whole-genome sequencing (short-read) were inconclusive.

In this study, NGS data was revisited by focusing on poorly covered, GC-rich ("dark") genomic areas, particularly on the X-chromosome (chrX), to potentially reveal unidentified clinically-relevant variants. Specifically, forty-three previously reported low-coverage chrX protein-coding regions were cross-checked against twenty-nine chrX genes/diseases highly associated ($p < 0.05$) with ID (HP:0001249) and focal dystonia (HP:0004373) according to the Phenomizer tool. The resulting regions were manually inspected with IGV to identify candidate variants. Finally, Sanger sequencing was used to confirm familial co-segregation of findings.

Two low-coverage regions resulted from the cross-check; chrX:25013469-25013696 and chrX:111744737-111744820 (hg38) overlapping the *ARX* (aristaless-related homeobox) and *ALG13* genes, respectively. The former was of particular interest as it overlaps a mutation hotspot associated with non-syndromic or syndromic X-linked ID, including the hand dystonia-related Partington syndrome. Visual inspection of whole-genome data revealed a recurrent *ARX* pathogenic variant NM_139058.3:c.441_464dup, p.Ala148_Ala155dup (*ARX*dup24), which was never flagged by downstream NGS analyses but was nevertheless confirmed in all affected males. The non-affected mothers were *ARX*dup24 carriers, while the non-affected fathers, other non-affected relatives, and healthy unrelated control were *ARX*dup24 negative.

In conclusion, the pathogenic *ARX*dup24 variant was unmasked supporting genotype-phenotype correlation in the first Partington syndrome family in Cyprus. Investigating the "dark" genome and utilizing Phenomizer for diagnostic assistance are highlighted to identify clinically-relevant variants in unsolved cases/families.

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P08.008.B Diagnosis in the era of NGS and variant reclassifications: a case of genetic disease or not?

Amelia Dobrescu[1], *Alexandru Caramizaru*[2], *Cristina Durac*[2], *Raluca Tutunaru*[2], *Andreea Catana*[3]

[1]CRGM Dolj, UMF Craiova, Craiova, Romania, [2]CRGM Dolj, Craiova, Romania, [3]UMF Iuliu Hatieganu, Cluj, Romania.

Introduction: In the past years, we have witnessed a remarkable technological evolution in genetic testing. However, from a clinician's point of view, it is still difficult to manage a patient with neuromotor developmental delay and to determine the genetic or non-genetic nature of the disease.

Materials and Methods: We report the case of a 10 year old patient who presented with a regression in neuromotor development at age one. Later on, further aspects emerged: ASD, ADHD, delayed motor and language development, intellectual and learning disability, generalized seizures, focal epilepsy, tic disorders and sleep problems. Multiple genetic investigations were performed.

Results: The karyotype was normal (46,XY), as was the number of CGG repeats. CGH-array showed a 391 kb gain on chromosome X, but the CNV was not considered pathogenic. WES identified two heterozygous variants in the ASXL2 gene, both classified as VUS, and the diagnosis of Sashi-Pena syndrome was possible. Three years later, after the family decided to perform a prenatal diagnosis for a new pregnancy, the diagnosis of Sashi-Pena syndrome for the index patient was also excluded due to the fact that the two ASXL2 variants were reclassified as likely benign.

Conclusions: A few questions remain unanswered regarding this case. Do the clinical aspects have a genetic cause? Is a test like whole genome sequencing needed? Could there also be a reclassification for the CNV discovered by CGH-array? Should we offer genetic counseling based on the information we have, or refrain and wait for more evidence to become available?

A. Dobrescu: None. **A. Caramizaru:** None. **C. Durac:** None. **R. Tutunaru:** None. **A. Catana:** None.

P08.009.C A new case of Bainbridge-Ropers syndrome

Ilona Jaszczuk[1], *Izabela Winkler*[2], *Agnieszka Sobczyńska-Tomaszewska*[3], *Aleksandra Pietrzyk*[3], *Monika Lejman*[4], *Wiktor Wojczakowski*[5], *Dorota Koczkodaj*[1]

[1]*Department of Cancer Genetics with Cytogenetic Laboratory, Medical University, Lublin, Poland,* [2]*St'Johns Center Oncology, Lublin, Poland,* [3]*MedGen Medical Centre, Warsaw, Poland,* [4]*Genetic Diagnostics, Department of Pediatric Hemathology, Oncology and Transplantology, Medical University, Lublin, Poland,* [5]*Department of Pediatric Hematology, Oncology and Transplantology, Lublin, Poland.*

Introduction: Bainbridge- Ropers syndrome (BRPS; OMIM # 615485) is a rare developmental disorder characterized by psychomotor and speech delays, intellectual disability and autism spectrum disorders, first described in 2013. Additional clinical features include hypotonia, feeding difficulties, postnatal growth failure and dysmorphic facial features. The underlying cause of the syndrome is constitutive variants in the *ASXL3* gene. We present an 10-old girl with recognized Bainbrigde- Ropers syndrome and confirmed variant in the *ASXL3* gene.

Materials and methods: During diagnostic procedure analysis of karyotyping, MLPA test (P-245), comparative genomic hybridization to microarray (aCGH) study and sequencing a panel of 372 genes (NGS) correlated with short stature, dysmorphic features and mental retardation were performed.

Results: The cytogenetic analysis showed normal balanced female karyotype 46,XX. Molecular analyses with MLPA and aCGH methods did not reveal any genome imbalances. NGS analysis allowed identification of new heterozygotic variant p.Glu367GlyfsTer17 (c.1095_1096delAA) in the *ASXL3* gene. This variant is reported in dbSNP database (rs1599562180) and ClinVar Database as likely pathogenic. Mutation was confirmed using Sanger sequencing. Molecular analysis of p.Glu367GlyfsTer17 was also performed for proband's parents but mutation was not identified, what confirmed *de novo* character of variant of *ASXL3* gene.

Conclusions: In the case of patients with intellectual disability and autism spectrum disorders, BRPS should be considered in the differential diagnosis. Research using the NGS technique facilitates and accelerates the diagnosis of patients with delayed psychomotor and speech development, ASD and dysmorphic features. Patients with BRPS require multidirectional care with the individualization of the learning process.

I. Jaszczuk: None. **I. Winkler:** None. **A. Sobczyńska-Tomaszewska:** None. **A. Pietrzyk:** None. **M. Lejman:** None. **W. Wojczakowski:** None. **D. Koczkodaj:** None.

P08.010.D *WAC* Related intellectual disability: presentation of five new patients

Clothilde ORMIERES[1], *Marlène RIO*[1], *Jeanne AMIEL*[1], *Severine DRUNAT*[2], *Sophie RONDEAU*[3], *Céline HUBER*[4], *Valérie CORMIER DAIRE*[1,4]

[1]*Service de Génétique clinique, AP-HP, Hôpital Necker Enfants Malades, Paris, France,* [2]*Service de génétique moléculaire, AP-HP, Hôpital Robert Debré, Paris, France,* [3]*Service de biologie moléculaire, AP-PH, Hôpital Necker Enfants Malades, Paris, France,* [4]*Université de Paris, Laboratory of Molecular and Physiopathological Bases of Osteochondrodysplasia, INSERM UMR 1163, Imagine Institute, Paris, France.*

Background: Deletions encompassing 10p11.23 and de novo variants in *WAC* are responsible for DeSanto-Shinawi syndrome, described for the first time in 2012 and reported less than 20 cases so far.

Methods: We report here the clinical and molecular characterization of 5 unrelated patients, 4 with loss-of-function WAC variants and 1 with a deletion encompassing 10p11.23. Clinical data were obtained by retrospective file analysis, clinics and formal neuropsychological evaluation.

Results: Clinical findings included hypotonia (4/5), developmental delay (5/5), intellectual disability (3/5), behavioral problems (3/5), eye abnormalities (5/5), sleep problems (2/5), attention deficit-hyperactivity (3/5), anxiety (5/5), MRI abnormalities (2/5), short stature (3/5), feeding difficulties (1/5), hypogammaglobulinemia (1/5) growth hormone deficiency (1/5), kidney abnormalities (1/5), cardiopathy (1/5). All patients have dysmorphic features. We identified one de novo deletion 60 kb 10p11.23 encompassing WACand four new heterozygous de novo *WAC* variants, including two nonsense, and two frameshift variants. All were predicted to cause loss of function either through nonsense-mediated mRNA decay or protein truncation.

Conclusions: Our series improves clinical description of WAC-related intellectual disability. Patients presented with recognisable characteristic facial dysmorphism and a variable neurocognitive phenotype. Interestingly intellectual disability was quite variable while behavioral and attention disorders were consistently observed. Functional studies are necessary to improve the understanding of the pathogenicity of *WAC* variants.

C. Ormieres: None. **M. Rio:** None. **J. Amiel:** None. **S. Drunat:** None. **S. Rondeau:** None. **C. Huber:** None. **V. Cormier daire:** None.

P08.011.A BICRA-based neurodevelopmental disorder: Two additional case reports and computational analysis of facial gestalt

Axel Schmidt[1], *Tzung-Chien Hsieh*[2], *Alexej Knaus*[2], *Sophia Peters*[1], *Elisabeth Mangold*[1], *Martina Kreiß*[1], *Janbernd Kirschner*[1], *Hartmut Engels*[1], *Peter M. Krawitz*[2]

[1]*University Hospital Bonn, Bonn, Germany,* [2]*University of Bonn, Bonn, Germany.*

Recently, de novo loss-of-function and missense variants in the gene BICRA were described as causative for a novel autosomal-dominant neurodevelopmental disorder (NDD) in twelve patients. Using trio exome sequencing in individuals with NDDs, we identified two children with rare heterozygous de novo loss-of-function BICRA variants (frameshift and nonsense). Child 1 was born small for gestational age and presents with microcephaly, speech delay, ectopic renal tissue and dysmorphic stigmata including epicanthal folds, a broad nasal bridge and upslanting palpebral fissures. Child 2 postnatally developed short stature with pronounced microcephaly, developmental delay, incomplete right bundle branch block, and facial dysmorphisms (broad nasal bridge, downslanting palpebral fissures, epicanthal folds). Of note,

child 2 also has a molecularly confirmed spinal muscular atrophy, which was treated by Nusinersen followed by Onasemnogene abeparvovec. To compare the facial phenotypes of the present patients and the seven published patients with available facial images, we applied the GestaltMatcher algorithm. This analysis revealed overall similarities between the patients with two noteworthy clusters of similar facial gestalts: one with two individuals (missense variant and small deletion) and one cluster with six individuals including our patients (frameshift, nonsense, and small deletion variants). In summary, the phenotypes of the two children reported here fit well with the features of the recently reported BICRA-based NDD such as developmental delays, microcephaly, short stature and genitourinary and cardiac abnormalities. In addition, GestaltMatcher successfully detected similarities of facial features between patients. Therefore, our report solidifies the BICRA-based NDD as a distinct disease entity.

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P08.012.B A homozygous truncating variant in *CCDC186* in an individual with epileptic encephalopathy

Melanie Brugger[1], *Fiona Becker-Dettling*[2], *Theresa Brunet*[1], *Tim Strom*[1], *Thomas Meitinger*[1], *Susanna Müller*[3], *Kristina Huß*[2], *Eberhard Lurz*[4], *Ingo Borggräfe*[2,5], *Matias Wagner*[1,6]

[1]*Institute of Human Genetics, School of Medicine, Technical University of Munich, Munich, Germany,* [2]*Division of Pediatric Neurology, Developmental Medicine and Social Pediatrics, Department of Pediatrics, Dr. von Haunersches Childrens Hospital, Ludwig Maximilians University of Munich, Munich, Germany,* [3]*Institute of Pathology, Ludwig Maximilians University of Munich, Munich, Germany,* [4]*Division of Pediatric Gastroenterology, Dr. von Haunersches Childrens Hospital, Ludwig Maximilians University of Munich, Munich, Germany,* [5]*Comprensive Epilepsy Center Ludwig Maximilians University of Munich, Munich, Germany,* [6]*Institute of Neurogenomics, Helmholtz Zentrum München GmbH, German Research Center for Environmental Health, Neuherberg, Germany.*

Introduction: A disease association of biallelic variants in *CCDC186*, a downstream effector of RAB2 involved in dense core vesicle trafficking, has been previously suggested, but only a single patient has been described in the literature. Previous studies in *C. elegans* and rat insulinoma cells illustrate the importance of CCDC186 (or its orthologue CCCP-1) for formation and cargo sorting of dense core vesicles in neurons, (neuro) endocrine and exocrine cells.

Material and Methods: We performed exome sequencing of a female patient presenting with epileptic encephalopathy and her parents at the Institute of Human Genetics, Technical University of Munich, Germany. Clinical investigations and treatment of the patient was performed at the Dr. von Haunersches Childrens Hospital in Munich, Germany.

Results: The 2-year old female patient born form consanguineous parents presented with severe developmental delay, microcephaly and epileptic encephalopathy with no ability to sit and lacking visual fixation of objects. Seizures and EEG were refractory to several antiepileptic drugs and steroids. Additionally, failure to thrive and feeding difficulties requiring a percutaneous feeding tube as well as exocrine and possible endocrine pancreatic dysfunction were present. Trio exome sequencing identified the homozygous loss-of-function variant c.767C>G; p.(Ser256Ter) in the candidate gene *CCDC186* (NM_018017.2).

Conclusions: We provide detailed clinical information on a patient with a biallelic truncating variant in *CCDC186* and illustrate the phenotypic similarities to the previously reported patient. Thus, we further strengthen an association of *CCDC186* biallelic variants with a severe neurodevelopmental disorder.

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P08.013.C CDC25B biallelic variants cause short stature, microcephaly, intellectual disability, developmental delay, facial dysmorphism and microphthalmia

Muhammad Ansar[1,2], *Fabienne Pituello*[3], *Sohail A. Paracha*[4], *Sophie Bel-Vialar*[3], *Emmanuelle Ranza*[2,5], *Federico A. Santoni*[2,6], *Muhammad T. Sarwar*[4], *Jawad Ahmed*[4], *Eric Agius*[3], *Stylianos E. Antonarakis*[2,7,8]

[1]*Institute of Molecular and Clinical Ophthalmology Basel (IOB), Basel, Switzerland,* [2]*Department of Genetic Medicine and Development, University of Geneva, Geneva, Switzerland,* [3]*Centre de Biologie du Developpement, Centre de Biologie Integrative, Universite de Toulouse, CNRS, UPS, Toulouse, France,* [4]*Institute of Basic Medical Sciences, Khyber Medical University, Peshawar, Pakistan,* [5]*Medigenome, Swiss Institute of Genomic Medicine, Geneva, Switzerland,* [6]*Department of Endocrinology Diabetes and Metabolism, University hospital of Lausanne, Lausanne, Switzerland,* [7]*Service of Genetic Medicine, University Hospitals of Geneva, Geneva, Switzerland,* [8]*iGE3 Institute of Genetics and Genomics of Geneva, Geneva, Switzerland.*

There are many rare autosomal recessive disorders for which the exact molecular etiology remains unknown. Consanguinity that results in large genomic regions of homozygosity facilitates the detection of novel gene-disease links. Here, we report three affected individuals (two siblings and one cousin) with short stature, microcephaly, severe intellectual disability, developmental delay, hypotonia, facial dysmorphism and microphthalmia, from a Pakistani consanguineous family in which we have identified homozygosity for p.(Arg350Pro) in the CDC25B gene (Genbank NM_021873.3) that segregated with the disease phenotype. CDC25B (Cell Division Cycle 25B), a member of the CDC25 family of phosphatases, is a tyrosine protein phosphatase, which induces the mitotic progression and activates the cyclin dependent kinase CDC2 by removing two phosphate groups. Experiments in chick embryos showed that the mutant protein (CDC25B with Pro350) affects the cell cycle and neurogenesis. The genetic evidence in the family and functional experiments in chick embryos indicate that the homozygous pathogenic variant in *CDC25B* are likely the cause of a recessive syndrome with short stature, microcephaly, severe intellectual disability and developmental delay.

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P08.014.D <New heterozygous mutation in CDK13 gene in a child with developmental delay, dysmorphic facial features, and congenital heart defect.>

Ruya M. Bafaqih, *Azhar Maghribi, Zohor Azher*

Medical genetics department, Faculty of Medicine, Umm al-qura university, Makkah, Saudi Arabia.

CDK13 is a protein-coding gene for a member of the cyclin-dependent kinases family[1]. Heterozygous pathogenic mutations in CDK13 are inherited in an autosomal dominant manner characterized by congenital heart defects, dysmorphic facial

features, and intellectual developmental disorder[2]. We present data relating to a child investigated for global developmental delay, intellectual disability, malformations of the heart and great vessels, autistic traits, and attention deficit hyperactivity disorder. The whole-exome sequencing test identified in the CDK13 gene a heterozygous variant p. (Asp855Glu). To our knowledge, this variant is not reported before, and it is absent in the general population in the control databases. In silico analysis, predict a deleterious effect of this variant. Based on the ACMG guidelines, this variant is classified as a likely pathogenic variant. There are no variants identified in other genes in this child; this gene can most likely explain this child's phenotype. To our knowledge, there are a few cases reported with a mutation in cdk13 with the same clinical feature. **References:1**- Greifenberg, A. K., D. Hönig, K. Pilarova, R. Düster, K. Bartholomeeusen *et al*., 2016 Structural and Functional Analysis of the Cdk13/Cyclin K Complex. Cell Rep 14**:** 320-331. **2**- Bostwick, B., 1993 CDK13-Related Disorder in *GeneReviews(®)*, edited by M. P. Adam, H. H. Ardinger, R. A. Pagon, S. E. Wallace, L. J. H. Bean *et al*. University of Washington, Seattle Copyright © 1993-2020, University of Washington, Seattle. GeneReviews is a registered trademark of the University of Washington, Seattle. All rights reserved., Seattle (WA).

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P08.015.A Inherited variants in *CHD3* demonstrate variable expressivity in Snijders Blok-Campeau syndrome

Jet van der Spek[1,2], *Joery den Hoed*[3,4], *Lot Snijders Blok*[1,2,3], *Alexander J. M. Dingemans*[1,2], *Dick Schijven*[3,4], *E. Martina Bebin*[5], *Stefanie Beck-Wödl*[6], *Gea Beunders*[7], *Natasha Brown*[8], *Melanie Brugger*[9], *Theresa Brunet*[9], *Philippe M. Campeau*[10,11], *Goran Čuturilo*[12,13], *Tobias B. Haack*[6], *Irina Hüning*[14], *Ralf A. Husain*[15], *Benjamin Kamien*[16], *Christina M. Lill*[14,17,18], *Janine Magg*[6], *Aleš Maver*[19], *Danielle C. Monteil*[20], *Charlotte W. Ockeloen*[1], *Christopher Richmond*[8], *Eva M. C. Schwaibold*[21], *Marleen E. H. Simon*[22], *Steffi Spranger*[23], *Tiong Tan*[8], *Michelle L. Thompson*[24], *Ella Wilkins*[8,25], *Marjolein H. Willemsen*[1], *Lisenka E. L. M. Vissers*[1,2], *Simon E. Fisher*[2,3], *Tjitske Kleefstra*[1,2]

[1]*Department of Human Genetics, Radboud University Medical Center, Nijmegen, Netherlands,* [2]*Donders Institute for Brain, Cognition and Behaviour, Nijmegen, Netherlands,* [3]*Language and Genetics, Max Planck Institute for Psycholinguistics, Nijmegen, Netherlands,* [4]*International Max Planck Research School for Language Sciences, Max Planck Institute for Psycholinguistics, Nijmegen, Netherlands,* [5]*University of Alabama at Birmingham, Birmingham, AL, USA,* [6]*Institute of Medical Genetics and Applied Genomics, University of Tübingen, Tübingen, Germany,* [7]*University Medical Center Groningen, Groningen, Netherlands,* [8]*Victorian Clinical Genetics Services, Victoria, Australia,* [9]*Institute of Human Genetics, School of Medicine, Technical University Munich, Munich, Germany,* [10]*CHU Sainte-Justine Research Center, Montreal, QC, Canada,* [11]*Sainte-Justine Hospital, University of Montreal, Montreal, QC, Canada,* [12]*University Children's Hospital Belgrade, Belgrade, Serbia,* [13]*Faculty of Medicine, University of Belgrade, Belgrade, Serbia,* [14]*Institute of Human Genetic, University of Lübeck, Lübeck, Germany,* [15]*Department of Neuropediatrics, Jena University Hospital, Jena, Germany,* [16]*King Edward Memorial Hospital, Subiaco, Australia,* [17]*Lübeck Interdisciplinary Platform for Genome Analytics, University of Lübeck, Lübeck, Germany,* [18]*Ageing Epidemiology Unit, School of Public Health, Imperial College London, London, United Kingdom,* [19]*Centre for Mendelian Genomics, Clinical Institute of Medical Genetics, UMC Ljubljana, Ljubljana, Slovenia,* [20]*Naval Medical Center Portsmouth, Portsmouth, VA, USA,* [21]*Institute of Human Genetics, Heidelberg University, Heidelberg, Germany,* [22]*Department of Medical Genetics, University Medical Center Utrecht, Utrecht, Netherlands,* [23]*Praxis für Humangenetik-Bremen, Bremen, Germany,* [24]*HudsonAlpha Institute for Biotechnology, Huntsville, AL, USA,* [25]*Murdoch Children's Research Institute, Victoria, Australia.*

The number of genetic diagnoses in individuals with neurodevelopmental syndromes has greatly increased over the past decade. Whereas *de novo* occurrence strongly supports pathogenicity of a variant in commonly used diagnostic pipelines for next generation sequencing, inheritance from a seemingly healthy parent generally downgrades it. However, variable penetrance and expressivity challenge this paradigm.

De novo CHD3 variants cause Snijders Blok-Campeau syndrome (SNIBCPS; MIM#618205). Here, we identified nineteen families with an inherited *CHD3* variant, likely explaining the proband's phenotype (12 predicted pathogenic missense and 7 predicted loss of function variants). We observed no difference between the phenotype of probands with *de novo* and inherited *CHD3* variants, including developmental delay/intellectual disability (100%), speech delay (100%) and facial dysmorphisms. Carrier parents had a milder or absent phenotype.

In addition to clinical phenotyping we performed several analyses. First, we established a facial analysis model and showed that facial characteristics of probands with an inherited *CHD3* variant significantly overlapped with those of *de novo* cases. Secondly, we used an Human Phenotype Ontology based computational comparison to objectively demonstrate the similarity between *de novo* and inherited cases. Thirdly, we found that healthy individuals with *CHD3* stop-gain variants have lowered *CHD3* transcript and CHD3 protein levels. Lastly, we evaluated the effect of rare *CHD3* variance on a population level, using UK biobank data.

Taken together, our data demonstrate variable expressivity for SNIBCPS, and exemplify that rare inherited variation in genes described with *de novo* variants in neurodevelopmental syndromes, can be causal due to yet underreported variable expressivity.

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P08.018.D Novel variant in *DDX3X* causes syndromic *DDX3X* related neurodevelopmental disorder

Kamilė Šiaurytė[1], *Kristina Grigalionienė*[2,3], *Algirdas Utkus*[2], *Aušra Matulevičienė*[2]

[1]*Vilnius University, Vilnius, Lithuania,* [2]*Department of Human and Medical Genetics, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, Vilnius, Lithuania,* [3]*Centre for Medical Genetics at Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania.*

Introduction: *DDX3X* related neurodevelopmental disorder (*DDX3X*-NDD) is a very rare entity, with less than 200 cases described in literature, caused by mutation in *DDX3X* gene (Xp11.4). Characteristic features of *DDX3X*-NDD include mild to severe intellectual disability, hypotonia, behavioral problems, movement disorders, dysmorphic features.

Materials and methods: A girl, 9 years of age, from unrelated parents, presents with severe intellectual disability, mostly absent speech, muscle hypotonia, stereotypical movements, ataxic gait, convergent strabismus, occasional seizures. Notable phenotypic features include prominent arched eyebrows, low nasal tip, thick upper lip, high and narrow palate, misaligned teeth, distal hypotrophy of leg muscles, hypermobile small joints, lax skin, pronounced signs of puberty. Girl also exhibits hyperactive behavior, episodes of unprovoked laughter. SNP-CGH assay and other screening tests showed no pathology.

Results: Exome sequencing revealed heterozygous frameshift variant leading to premature stop codon NM_001356.5: c.1629_1630dupAT (NP_001347.3:p.(Phe544TyrfsTer8) in *DDX3X* gene. This variant was not reported in population databases (1000G, ExAC, GnomAD) and literature previously. Variant is classified as pathogenic in Varsome database and appears highly deleterious after *in silico* analysis.

Conclusion: The frameshift variant in *DDX3X* gene should be considered as a cause for intellectual disability in girls. However, characteristic features of *DDX3X*-NDD are unspecific and hardly distinguishable from other intellectual disability syndromes, posing challenge to clinicians. Exome sequencing is an indispensable tool to reach the final diagnosis and gather further evidence on very rare disorders.

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P08.019.A Gene spectrum in Russian patients with developmental delay, and/or epilepsy, and/or microcephaly via next-generation sequencing

***Victoria Zabnenkova**, Tatiana Cherevatova, Anna Orlova, Polina Gundorova, Olga Mironovich, Oxana Ryzhkova*

Research Centre for Medical Genetics, Moscow, Russian Federation.

Background: The diagnosis of developmental delay, epilepsy and microcephaly is complicated by the variability of the phenotypic manifestation. Patients may have combined dysmorphic features, intellectual disability, and seizures. NGS is an effective strategy for genetic analysis in this complex condition.

Materials and methods: DNA samples of 501 patients with developmental delay, and/or epilepsy, and/or microcephaly have been analyzed with WES(265, IDT) and CES(236, Roche).

Results: Pathogenic and likely pathogenic variants were identified in 84 genes in 107 patients (107/501, 21,4%). 14 genes have met two or more times: *SCN1A*, *ARID1B*, *AP4B1*, *ATP1A3*, *ANKRD11*, *CDKL5*, *IQSEC2*, *KIF11*, *KMT2D*, *LZTR1*, *MECP2*, *NALCN*, *POG2*, *SMARCA2*. Interestingly, the c.1160_1161delCA(p.Thr387ArgfsTer30) mutation was found three times in a hemi/homozygous state in the *AP4B1* gene(NM_006594.5), previously described as cause of spastic paraplegia (OMIM 614066). Also, there were two different mutations affecting the same codon 1181 in the *NALCN* gene(NM_052867.4). According to the type of inheritance the findings were divided as follows: 4 cases - X-linked, 30 cases - autosomal recessive, 73 cases - autosomal dominant (including *de novo*). Promising variants of uncertain significance are registered in 138 genes. There is a group of genes encoding proteins of ion channels (*KCN1*, *KCNB1*, *KCNE2*, *KCNH2*, *KCNJ10*, *KCNQ3*, *KCNT1*, *SCN2A*, *SCN3A*, *SCN8A*, *SCN9A*) in these findings. Variants occur not only in patients with epilepsy, but also in those with developmental delay due to epileptic encephalopathy.

Conclusion: The results of this study demonstrate significant genetic heterogeneity among patients with developmental delay, epilepsy, microcephaly, identify additional phenotypes, and expand the mutation spectrum.

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P08.020.B With or without highly restricted-Down syndrome critical region (HR-DSCR). report of two new partial trisomy 21 cases and review of the recent literature

***Maria Chiara Pelleri**[1], Chiara Locatelli[2], Teresa Mattina[3], Maria Clara Bonaglia[4], Francesca Piazza[1], Pamela Magini[5], Francesca Antonaros[1], Giuseppe Ramacieri[1], Beatrice Vione[1], Lorenza Vitale[1], Marco Seri[6], Pierluigi Strippoli[1], Guido Cocchi[7], Allison Piovesan[1], Maria Caracausi[1]*

[1]Department of Experimental, Diagnostic and Specialty Medicine (DIMES), University of Bologna, Bologna, Italy, [2]Neonatology Unit, St. Orsola-Malpighi Polyclinic, Bologna, Italy, [3]Medical Genetics Unit, University of Catania, Catania, Italy, [4]Cytogenetics Laboratory, Scientific Institute, IRCCS Eugenio Medea, Bosisio Parini (Lecco), Italy, [5]Medical Genetics Unit, St. Orsola-Malpighi Polyclinic, Bologna, Italy, [6]Medical Genetics Unit, St. Orsola-Malpighi Polyclinic, Department of Medical and Surgical Sciences (DIMEC), University of Bologna, Bologna, Italy, [7]Neonatology Unit, St. Orsola-Malpighi Polyclinic, Department of Medical and Surgical Sciences (DIMEC), University of Bologna, Bologna, Italy.

Introduction: Down syndrome (DS) is caused by the presence of an extra copy of full or partial human chromosome 21. Partial trisomy 21 (PT21) is an invaluable model for the study of genotype-phenotype correlation in DS. In particular, a 34-kb highly restricted DS critical region (HR-DSCR) has been identified as the minimal region whose duplication is shared by all PT21 subjects diagnosed with DS, while it is absent in all PT21 non-DS subjects reported in the literature up to 2017.

Materials and Methods: We report clinical data and cytogenetic characterizations of two PT21 children never described before. Moreover, we performed a systematic bibliographic search for new PT21 reports recently published to further investigate the association between the presence of three copies of the HR-DSCR and the DS diagnosis.

Results: Clinical and cytogenetic analyses of the two PT21 children reported here revealed specular features: one case with the HR-DSCR among the duplicated regions is diagnosed with DS, while the other without the HR-DSCR duplication has no DS diagnosis. As demonstrated by clinical data and cytogenetic map correlation also including PT21 reports recently published, we confirmed the presence of duplicated HR-DSCR in all DS subjects and its absence in all non-DS individuals.

Conclusions: The results are fully consistent with the hypothesis that the HR-DSCR is critically associated with DS diagnosis. No exception to this pathogenetic model was found. Further studies are needed to detect genetic determinants likely located in the HR-DSCR and possibly responsible for core DS features, in particular intellectual disability.

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P08.023.A Molecular analysis of a novel donor splice site variant in *DYNC1H1*

***Gunda Petraitytė**, Živilė Maldžienė, Violeta Mikštienė, Evelina Siavrienė, Tautvydas Rančelis, Vaidutis Kučinskas, Eglė Preikšaitienė*

Department of Human and Medical Genetics, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, Vilnius, Lithuania.

Introduction: Cytoplasmic dynein 1 is a cytoskeletal motor transporting various cargos in cells and playing specific roles such as empowering neurotrophic signaling important for neuronal survival. *DYNC1H1* (MIM#600112) gene encodes a heavy chain 1 of the cytoplasmic dynein. Heterozygous mutations of this gene are linked to neuromuscular (MIM#158600, MIM#614228) and neurodevelopmental disorders (MIM#614563). Here we describe a study performed to investigate the pathogenicity of a novel intron variant in *DYNC1H1*.

Materials and Methods: A male, 14 years of age, was referred for genetic assessment for intellectual disability and abnormality of cerebral cortex. WES was applied to analyse DNA of the proband and both parents. The DNA of healthy brother was analysed by Sanger sequencing. To determine the effect of identified splice site variant on mRNA structure, total blood mRNA of the proband was isolated, template cDNA was synthesized, and Sanger sequencing was performed.

Results: Analysis of DNA samples revealed heterozygous donor splice site variant NC_000014.9(NM_001376.5):c.6405+1G>C in *DYNC1H1* gene as *de novo* in the proband's DNA. *In silico*, this altered donor site probably affects mRNA splicing. PCR of proband's cDNA resulted in two different fragments. Sanger sequencing revealed retaining intron 31 in one of them, presumably leading to a frameshift and premature stop codon truncating motor region of the protein (NP_001367.2:p.(Ile2136LeufsTer20)).

Conclusions: The molecular analysis of the donor splice site variant NC_000014.9(NM_001376.5):c.6405+1G>C in *DYNC1H1* revealed disrupted mRNA splicing which leads to truncated and probably dysfunctional protein causing neurodevelopmental phenotype of the proband.

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P08.024.B Development delay in paediatric patient with deletion on chromosome 15q26.2

Milica Pesevska[1], *Violeta Anastasovska*[1], *Elena Sukarova-Angelovska*[1], *Dragica Nestoroska*[1], *Gordana Ilieva*[1], *Sandra Panovska*[2]

[1]*Genetic Laboratory, University Clinic for Pediatrics, Ss.Cyril and Methodius University in Skopje, Faculty of Medicine, Skopje, Macedonia, The Former Yugoslav Republic of,* [2]*Department of Human Genetics, Avicena Diagnostic, Skopje, Skopje, Macedonia, The Former Yugoslav Republic of.*

Introduction: Subtelomeric chromosomal regions are gene rich. Approximately 2.5% of the patients with mental retardation with or without association of dysmorphic features have deletions in these segments.

Materials and Methods: We performed a aCGH analysis in paediatric patient using the CytoScan_750k Array platform (Affymetrix) which comprises 550 k non-polymorphic and 200 k SNP markers by the Chromosome Analysis Suite (ChAS) Software (v4.0).

Results: We present a 4-year old girl with following dysmorphic signs - downward corners of the mouth and large oral opening, saddle nose with wide nasal root, retracted eye bulbs, triangular pointed eyebrows, asymmetrical placement of the eyelid, smaller opening of the lid, asthenic pointed forehead, short philtrum, small chin and sparse hair. She had development delay with fine rough motoric skills without significant hypotonia and signs of hyperactivity with poor vocabulary. Height and weight were under 3th percentile. Karyotype analysis was normal as well as FISH analysis for 4p- deletion performed by suspicions for Wolf-Hirschorn syndrome. aCGH analysis showed pathological deletion of 5,025 kb segment on 15q26.2 (14 OMIM genes) and additional duplication of 4,179 kb segment on the 1p36.33 chromosome (57 OMIM genes) according to ClinVar and OMIM database. The genes IGFR1, MEF2A, CHSY1, and TM2D3 in the deleted region could be delineated according to patient phenotype.

Conclusions: The deletion on 15q26.2 may lead to the description of clinically recognizable syndrome associated with development delay. Further examinations should be performed for including other genes in the pathological condition.

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P08.025.C Solve-RD: the ITHACA perspective

A-S Denommé-Pichon[*1,2], *E. de Boer*[*3,4], *A Jackson*[*5], *E Benetti*[*6], *S Banka*[5,7], *G Casari*[8,9], *A Ciolfi*[10], *J Clayton-Smith*[5,7], *B Dallapiccola*[10], *K Ellwanger*[11,12], *L Faivre*[2,13], *C Gilissen*[3,14], *H Graessner*[11,12], *T B. Haack*[11], *A Hammarsjö*[15], *M Havlovicova*[16], *A Hoischen*[3,14,17], *A Hugon*[18], *T Kleefstra*[3,4], *A Lindstrand*[15], *E López-Martín*[19], *M Macek Jr.*[16], *L Matalonga*[20], *M Morleo*[9], *V Nigro*[9,8], *A Nordgren*[15], *M Pettersson*[15], *M Pinelli*[9], *S Pizzi*[10], *M Posada*[19], *F C. Radio*[21], *A Renieri*[6,22,23], *C Rooryck*[24], *L Ryba*[16], *M Schwarz*[16], *M Tartaglia*[10], *C Thauvin*[1,2,13], *A Torella*[8,9], *A Trimouille*[25], *P Votypka*[16], *K Vyshka*[18,26], *B Zurek*[11,12], *A Verloes*[18,27], *A Vitobello*[*1,2], ***Lisenka Vissers***[*3,4]

[1]*Unité Fonctionnelle d'Innovation diagnostique des maladies rares, FHU-TRANSLAD, CHU Dijon Bourgogne, Dijon, France,* [2]*UFR Des Sciences de Santé, INSERM-Université de Bourgogne UMR1231 GAD « Génétique des Anomalies du Développement », FHU-TRANSLAD, Dijon, France,* [3]*Dept of Human Genetics, Radboudumc, Nijmegen, Netherlands,* [4]*Donders Institute for Brain, Cognition and Behaviour, Nijmegen, Netherlands,* [5]*Manchester Centre for Genomic Medicine, University of Manchester, Manchester, United Kingdom,* [6]*Med Biotech Hub and Competence Center, Dept of Medical Biotechnologies, University of Siena, Siena, Italy,* [7]*Manchester Centre for Genomic Medicine, Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom,* [8]*Dipartimento di Medicina di Precisione, Università degli Studi della Campania "Luigi Vanvitelli", Napoli, Italy,* [9]*Telethon Institute of Genetics and Medicine, Pozzuoli, Italy,* [10]*Genetics and Rare Diseases Research Division, Ospedale Pediatrico Bambino Gesù, IRCCS, Rome, Italy,* [11]*Institute of Medical Genetics and Applied Genomics, University of Tübingen, Tübingen, Germany,* [12]*Centre for Rare Diseases, University of Tübingen, Tübingen, Germany,* [13]*Dept of Genetics and Centres of Reference for Development disorders and intellectual disabilities, FHU TRANSLAD and GIMI Institute, University Hospital Dijon, Dijon, France,* [14]*Radboud Institute for Molecular Life Sciences, Nijmegen, Netherlands,* [15]*Dept of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden,* [16]*Dept of Biology and Medical Genetics, Charles University Prague, Prague, Czech Republic,* [17]*Dept of Internal Medicine and Radboud Center for Infectious Diseases, Radboudumc, Nijmegen, Netherlands,* [18]*Dept of Genetics, Assistance Publique-Hôpitaux de Paris - Université de Paris, Paris, France,* [19]*Institute of Rare Diseases Research, Spanish Undiagnosed Rare Diseases Cases Program & Undiagnosed Diseases Network International, Instituto de Salud Carlos III, Madrid, Spain,* [20]*CNAG-CRG, Centre for Genomic Regulation, The Barcelona Institute of Science and Technology, Barcelona, Spain,* [21]*Genetics and Rare Diseases Research Division, Bambino Gesù Children's Hospital, IRCCS, Rome, Rome, Italy,* [22]*Medical Genetics, University of Siena, Siena, Italy,* [23]*Genetica Medica, Azienda Ospedaliero-Universitaria Senese, Siena,*

Italy, [24]University Bordeaux, MRGM INSERM U1211, CHU de Bordeaux, Service de Génétique Médicale, Bordeaux, France, [25]Laboratoire de Génétique Moléculaire, Service de Génétique Médicale, CHU Bordeaux – Hôpital Pellegrin, Bordeaux, France, [26]CERCRID, UMR 5137 "Centre de Recherches Critiques en Droit", Université de Lyon, Lyon, France, [27]INSERM UMR 1141 "NeuroDiderot", Hôpital R DEBRE, Paris, France.

Solve-RD is a Horizon2020 supported EU flagship project aiming to solve rare diseases for which a molecular cause is not yet established. European Reference Network (ERN) Intellectual disability, TeleHealth And Congenital Anomalies (ERN-ITHACA) is one of four core ERNs contributing to this project. Here we describe our achievements so far.

(i) Unsolved exome negative patients

Over 5,200 data sets consisting of phenotypic and genomic information have been internationally shared in the Genome-Phenome Analysis Platform for systematic re-analysis. This has resulted in ~6-8% of novel diagnoses. Additionally, for 9 candidate disease genes, Solve-RD researchers have been matched with functional scientists via 'Rare Diseases: Models and Mechanisms (RDMM)'-Europe to study the molecular mechanism of disease.

(ii) ITHACA-specific cohorts

We are collecting genetically unsolved patients for the following syndromes: Moebius/Poland, Goldenhar, Wildervanck, Baraitser-Winter, primary microcephalic dwarfism, MURCS, RAS-opathies and 'Rett-like'-phenotypes. For these cohorts, the first short-read genomes are being performed.

(iii) Ultra-rare patients

All ~80 Health Care Providers in ITHACA are collecting (ultra-) rare unsolved patients. Though phenotypic jamborees, 100 families are selected for WGS.

(iv) Unsolvable syndromes

We aim to unravel the genetic etiology of Aicardi, Gomez-Lopez-Hernandez, Pai, OAFNS and Hallermann-Streiff syndrome. Hereto, a multi-omics approach is planned for, including long-read genomes, transcriptomics, epigenomics, deep-WES and metabolomics on multiple tissues per patient-parent trio. First analyses are ongoing.

The Solve-RD project has enabled ITHACA data sharing and clinical patient selection at a pan-European level. We expect to facilitate diagnoses for unsolved patients, and to elucidate the molecular underpinning of ITHACA-related unexplained syndromes.

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P08.026.D Contiguous gene deletion of *MSH6* and *FBXO11*. Presenting a large family with Lynch syndrome and *FBX011*-related intellectual developmental disorder

Malene Lundsgaard[1], *Uffe B. Jensen*[2], *Anja Ernst*[3], *Henrik Okkels*[3], *Rikke Christensen*[2], *Thomas Balslev*[4], *Lone Sunde*[1], *Charlotte K. Lautrup*[1,2]

[1]Department of Clinical Genetics, Aalborg University Hospital, Aalborg, Denmark, [2]Department of Clinical Genetics, Aarhus University Hospital, Aarhus, Denmark, [3]Department of Molecular Diagnostics, Aalborg University Hospital, Aalborg, Denmark, [4]Department of Paediatrics, Viborg Regional Hospital, Viborg, Denmark.

Introduction: *MSH6* and *FBXO11* are located at chromosome 2p16.3 with overlapping 3' UTR ends by 33 bp. *MSH6*-related Lynch syndrome is well-described however fewer than 50 patients with *FBXO11*-related intellectual developmental disorder with dysmorphic facies and behavioral abnormalities (IDDFBA) have been reported. Most represent *de novo* cases detected by trio-whole exome sequencing (trio-WES). Only one familial *FBXO11* case has been reported. We present a large family with a 0,1Mb deletion involving *MSH6* and the 3' UTR end of *FBXO11*.Clinical findings: The proband is a three year old boy with speech- and motor delay, hypotonia, low-set ears with a closed helix, thin upper lip vermillion, long philtrum and paternal family history of both intellectual disability and Lynch syndrome caused by deletion of *MSH6*. Chromosomal microarray revealed the familial 0,1Mb deletion on 2p16.3 involving *MSH6* and trio-WES was without pathogenic variants.

Results: Review of the family revealed that 7/8 family members with the deletion had variable degrees of learning disabilities and 3/8 had psychiatric disturbances. The proband's gestalt was similar to that reported in IDDFBA patients; however, the features were not consistent in relatives. Revisiting chromosomal microarray data indicated that deletion involves the terminal 3' UTR of *FBXO11*, confirmed by CNV analysis of the onco-gene panel (SWEBRCA).

Conclusion: The terminal deletion of *FBXO11* most likely explains the IDDFBA-phenotype seen in this family. Due to limitations in trio-WES analyses, a small CNV might be missed and an inherited *FBXO11* variant will also be missed when searching for *de novo* variants.

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P08.028.B Electrophysiological and proteomic investigations of an Ftsj1-deficient mouse model for intellectual disability

***Lars Jensen**, Marian Baldus, Simone Venz, Heike Junker, Viola von Bohlen und Halbach, Oliver von Bohlen und Halbach, Andreas Kuß*

University Medicine Greifswald, Greifswald, Germany.

Inherited intellectual disability (ID) is a genetically very heterogeneous disorder. Though mutations in each gene are relatively rare, several genes involved in charging and modifications of tRNA molecules have been found mutated in ID-patients. tRNAs transport amino acids to the ribosome but are also involved in a variety of other cellular processes. The underlying molecular causes for the observed ID phenotype is at present not well understood. Inactivating FTSJ1 mutations have been found in several families with non-syndromic ID. FTSJ1 is an evolutionarily conserved, ubiquitously expressed tRNA-methyltransferase that targets a subset of tRNAs. We have previously established a mouse model for FTSJ1 deficiency and found altered energy metabolism, increased pain threshold in addition to learning defects in Ftsj1 deficient mice. To investigate neuronal function at the cellular level we now performed electrophysiological experiments, inducing long-term potentiation (LTP) in hippocampal neurons from mutant and wild-type mice. We observed significantly weaker LTP in Ftsj1-deficient animals than in control littermates. We then used

protein extracts from whole brain preparations to generate protein abundance profiles using 2D-PAGE coupled with mass spectrometry for peptide identification and identified peptides corresponding to 25 unique proteins with >1.5-fold difference in abundance, including alpha-Tubulin. Functional annotation analysis of these proteins showed, among others, enrichment for the GO term "cytoskeletal protein binding". Our results suggest that Ftsj1 deficiency leads to a loss of synaptic strength in hippocampal neurons, which may contribute centrally to the ID phenotype observed in FTSJ1 deficient individuals and pinpoints interesting novel functional aspects of FTSJ1.

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P08.029.C Next generation sequencing of a genes panel for the study of Mexican patients with global developmental delay

María de la Luz Arenas-Sordo[1,2], *Paola Linares-Mendoza*[1], *Karina Peñuelas-Romero*[1], *Javier Martínez-Martínez*[1]

[1]*Instituto Nacional de Rehablitacion, Mexico City, Mexico,* [2]*Universidad Nacional autónoma de México, México City, Mexico.*

Introduction: GDD is defined as a significant delay in 2 of the major developmental domains (gross/fine motor,speech/language, cognition, social/personal, and activities of daily living), although most affected patients have impairment evident in all 5 of the domains. The diagnosis is reverved for children under 5 years og age. The prevalence estimates between 1 and 3% of children. Around 25-50% of GDD cases can be secondary to genetic causes.

Materials and Methods: An exome of 506 genes was done in 20 patients, after discarded other causes through karyotype, metabolic screening, and MRI. The patients were studied with detail in their phenotype and with a neurological evaluation.

Results: Of the 20 patients studied, 14 were female and 6 male. None reported consanguinity and just 1 had inbreeding. One patient has a brother with the same condition. Some of them have dysmorphia, short stature, epilepsy, microcephaly, deafness, etc. Metabolic screening and karyotype were normal in all patients. Some MRI showed not specific alterations. The exome found variants in: *TYR, ZNF423, ALDH18A1, POMPT1 and CA6*, all in heterozigous way. Also a CNV's in the NSUN2 gene that caused deletion of 33.39 kb (chr.5) and one non-related finding, variant in G6PD gene (c.934G>C; p.Asp312His).

Conclusions: In these preliminary results, we found a deletion in the NSUN2 gene as the cause of GDD and the other genes could be related with it. It is necessary to study the other variants that we found, in the multiple existing databases and make some predictions with different computer systems.

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P08.031.A Genotype-phenotype effects of the X-linked *GSPT2* gene: severe developmental and epileptic encephalopathy in patients with missense variants versus mild developmental delay in patients with deletions

Gea Beunders[1], *Evert Kas*[1], *Sue White*[2,3], *Kristin M. Abbott*[1], *Yvonne J. Vos*[1], *Cleo C. Diemen*[1]

[1]*UMCG, Groningen, Netherlands,* [2]*University of Melbourne, Melbourne, Australia,* [3]*Victorian Clinical Genetics Service, Melbourne, Australia.*

Introduction: Recently, a new x-linked intellectual disability syndrome was described, caused by deletions of Xp11.22 with the smallest region of overlap including *CENPVL1*, *CENPVL2*, *MAGED1* and *GSPT2*. *GSPT2* was suggested as the causal gene for the intellectual disability. *GSPT2* encodes eRF3b that, together with eRF3a (encoded by *GSPT1*), is forming a complex with eRF1. This complex is composing a crucial role in translation termination and nonsense mediated decay.

Patients & methods: We describe family one, highly suggestive of X-linked inheritance, with three affected boys and two obligate carriers (unaffected females); and family two, with one affected boy. The overlapping clinical features in the four affected boys are: central and obstructive apneas, severe epilepsy, profound developmental delay, a secondary microcephaly, mild a-specific dysmorphic features and edema. The three boys from family one died in infancy. Whole Exome Sequencing was performed in the two index patients.

Results: Missense variants in the GTP-ase domain of *GSPT2* were found in both index patients. In family one, the variant segregated with the phenotype and was found in two affected boys, two carrier females and was not found in one unaffected male.

Conclusions: *GSPT2* missense variants seem to cause a severe x-linked epileptic encephalopathy, whereas larger deletions including *GSPT2* cause a milder intellectual disability. We hypothesize that the missense variants described here cause an altered eRF3 complex that hampers the translation termination and/or nonsense mediated decay more than a deletion of the gene does. Functional studies are underway to proof this hypothesis.

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P08.032.B Mosaic copy number gain chr1p35.1p33 causing a severe phenotype with intellectualdisability and macrocepahly

Maria Puiu[1], *Alexandra Mihailescu*[2], *Adela Chirita-Emandi*[1]

[1]*Center of Genomic Medicine, University of Medicine and Pharmacy "Victor Babes", Regional Center of Medical Genetics Timis, Clinical Emergency Hospital for Children "Louis Turcanu", part of ERN ITHACA, TIMISOARA, Romania,* [2]*Center of Genomic Medicine, University of Medicine and Pharmacy "Victor Babes", TIMISOARA, Romania.*

Duplications involving the 1p35p33 chromosomal region are very rare, associating global developmental delay, craniofacial dysmorphism, heart malformations and growth failure.

Material: A 3-year-old girl, presenting with hypotonia, global developmental delay, macrocephaly, frontal bossing, opened anterior fontanelle, flat nasal bridge, hypertelorism, ptosis, prognathism, spaced teeth, bilateral simian crease, clinodactyly, cavovarus, large head and chest compared to lower body, ataxic gait, no language, behavioral issues (stereotypes, anxiety, hand flapping, bruxism) and slow weight gain.

Results: She was diagnosed with atrial septal defect. Brain MRIs at age 1 year and 3 years revealed normal brain structure, without hydrocephalus/ventriculomegaly. SNP array: [GRCh37] chr1p35.1p33(33920586_50612250) x3[0.7] showed 16,7 Mb copy number gain, mosaic clone 70%. The copy number gain was de novo. The region contains several autosomal dominant transmission genes: GJB3, KCNQ4 associated with neurosensory deafness, COL9A2 - epiphyseal dysplasia, COL8A2 - corneal dystrophy/ aortic malformations, SLC2A1 - Dystonia/epilepsy. However, the implications of copy number gainof these genes cannot be accurately assessed. Only 2 patients with similar gains are reported in

decipher.sanger.ac.uk(ID:353680;401008).One of these patients also presented polydactily.

Conclusion: Pathogenicity in copy number gains are difficult to understand. Evenmore if the number of patients reported is very small and mosaic, multicentric collaboration is crucial for understanding the phenotype and prognosis.

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P08.034.D DDX3X syndrome phenotype heterogeneity a case report and literature review

Rossella Colantuono[1], *Maria Chiara Rocco*[1], *Dario Di Salvio*[2], *Maria Tessitore*[1], *Lucia Nazzaro*[3], *Domenico Viggiano*[1], *Maria Teresa Falco*[3], *Giorgia Mancano*[4], *Tjitske Kleefstra*[5], *Lot Snijders Blok*[5], *Matteo Della Monica*[6], *Pietro Vajro*[1], *Daniela Melis*[1]

[1]*Pediatrics, Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, Baronissi (SA), Italy,* [2]*Pediatrics, University of Naples "Federico II", Napoli, Italy,* [3]*"San Giovanni di Dio e Ruggi d'Aragona" University Hospital, Salerno, Italy,* [4]*Meyer University Hospital, Firenze, Italy,* [5]*Department of Human Genetics, Radboud University Medical Center, Nijmegen, Netherlands,* [6]*Department of Medical and Laboratory Genetics, Cardarelli Hospital, Naples, Italy.*

About 25-50% of intellectual disability (ID) is genetically determined, and X-linked ID (XLID) is a major pathogenic cause. *De novo* mutations of the DDX3X gene account for 1-3% of ID in females. The 3q29 duplication has a quite overlapping phenotype and low penetrance, possibly influenced by "second hit" genetic aberrations.

Case report: We describe a normal height, slightly obese female patient with ID, language impairment, facial dysmorphisms, macrocephaly, scoliosis, skin abnormalities, brain abnormalities (septum pellucidus and cavum vergae cysts), hepatosplenomegaly, hypertransaminasemia and hepatic steatosis. Exome sequencing identified in the patient a heterozygous *de novo* pathogenic variant (c.1463G> A; p.Arg488His) in *DDX3X* gene. A 229 Kb 3q29 microduplication, not overlapping the critical region for 3q29 duplication syndrome, was previously detected by CGH array in the patient and her healthy mother.

Literature Review: review of ID cases caused by DDX3X variants and 3q29 duplications to compare their genetic and phenotypic spectra vs. our patient.

Results: Microcephaly and low weight were often associated with the DDX3X spectrum, whereas obesity, hepatic involvement and macrocephaly were reported. The same DDX3X variant of our patient was previously reported only in one case with some phenotype differences (low weight, corpus callosum hypoplasia, ventricular enlargement, cleft lip/palate). Microduplications of 3q29 may present generalized obesity.

Conclusions: We report a patient with DDX3X variant and atypical phenotype. Clinical variability could be explained by a "second hit" genetic aberration and we suppose a possible role of the 3q29 microduplication previously detected in the patient and her mother, thus hampering correct categorization.

R. Colantuono: None. **M. Rocco:** None. **D. Di Salvio:** None. **M. Tessitore:** None. **L. Nazzaro:** None. **D. Viggiano:** None. **M. Falco:** None. **G. Mancano:** None. **T. Kleefstra:** None. **L. Blok:** None. **M. Della Monica:** None. **P. Vajro:** None. **D. Melis:** None.

P08.035.A Multiple major anomalies and microcephaly predict the detection of pathogenic copy number variations in patients with moderate and severe global developmental delay/intellectual disability

Jelena Ruml Stojanovic[1], *Marija Mijovic*[1], *Aleksandra Miletic*[1], *Brankica Bosankic*[1], *Hristina Petrovic*[1], *Goran Cuturilo*[1,2]

[1]*University Children's Hospital, Belgrade, Serbia,* [2]*Faculty of Medicine, University of Belgrade, Belgrade, Serbia.*

Introduction: Moderate and severe forms of intellectual disability and global developmental delay are genetically and clinically heterogeneous entities. Evaluation of genetic cause is often challenging, and many patients undergo a "diagnostic odyssey". Defining the clinical parameters in correlation with pathogenic copy number variations could enable better selection of patients for chromosomal microarray analysis (CMA) and speed up the diagnostic process.

Materials and Methods: The present study included 110 children with the diagnosis of moderate or severe global developmental delay/intellectual disability, present either solely or with diverse additional findings. CMA was performed in all patients. Correlation of clinical parameters and CMA results was assessed using Pearson chi-square and Fisher's Exact tests.

Results: Pathogenic/likely pathogenic variants were identified in 26,4% (29/110) of patients, variants of uncertain significance in 12,7% (14/110) of patients, while the rest had normal result or benign variant identified (60,9%, 67/110). Statistical analysis revealed that patients with multiple congenital anomalies and/or microcephaly were more likely to have pathogenic copy number variation identified.

Conclusion: There were several attempts in literature to define clinical parameters that could predict the presence of pathogenic CMA result in patients with global developmental delay/intellectual disability and the results are inconsistent. Present data suggest CMA as a method of choice in children with multiple congenital anomalies and/or microcephaly. Study with a larger number of patients, or meta-analysis of previously performed studies, could give more precise insight in correlation of phenotypic features and pathogenic CMA results in this specific group of patients.

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P08.036.B High diagnostic yield using a 460 gene panel in patients with intellectual disability

Nino Spataro[1], *Miriam Guitart*[1], *Elisabeth Gabau*[2], *Nuria Capdevila*[2], *Juan Pablo Trujillo-Quintero*[2], *Laura Capel*[1], *Neus Baena*[1], *Anna Ruiz*[1]

[1]*Genetics Laboratory, UDIAT-Centre Diagnòstic. Parc Taulí Hospital Universitari. Institut d'Investigació i Innovació Parc Taulí I3PT. Universitat Autònoma de Barcelona, Sabadell, Spain,* [2]*Paediatric Unit. ParcTaulí Hospital Universitari. Institut d'Investigació i Innovació Parc Taulí I3PT. Universitat Autònoma de Barcelona, Sabadell, Spain.*

Introduction: Intellectual disability (ID) affects 1-3% of the population and is defined by deficits in intellectual functioning and adaptive behavior with onset before age 18 years. Approximately, 70% of ID cases are due to genetic factors, among those de novo pathogenic variants in dominant genes account for the majority of cases.

Material and methods: We developed a dominant and X-linked ID gene panel including 460 genes. The panel was tested

on 223 patients affected by ID and with negative aCGH. Sequencing was performed in an Illumina MiSeq System; a customised bioinformatic pipeline was developed for discovery, annotation and filtering of SNVs, indels and CNVs. Sanger sequencing on parental samples was used for segregation analysis of candidate variants.

Results: A diagnostic yield of 30,9% was obtained after target high depth sequencing. 74,2% of pathogenic variants were de novo while 15% were inherited, of which 50% were in X-linked genes. For the remaining pathogenic variants segregation analysis was not possible. The implemented bioinformatic pipeline allows a high detection rate of pathogenic variants minimizing the detection of variants of unknown significance.

Conclusion: The diagnostic yield of our customised ID panel is higher than that of aCGH. We propose the use of our ID panel as first tier diagnosis in unexplained ID patients. Even though exome sequencing may yield a higher diagnostic rate, the use of an ID panel is cost effective, time saving and minimize the detection of variants of unknown significance.

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P08.037.C *THUMPD1* is a new cause of syndromic intellectual developmental disorder

Martin Broly[1], *Bertrand Isidor*[1,2], *Thomas Besnard*[1,2], *Declan O'Rourke*[3], *Julia Baptista*[4,5], *Sian Ellard*[4,5], *Mohammed Almannai*[6], *Hashem Mais Omar*[7], *Ferdous Abdulwahab*[7], *Hanan Shamseldin*[7], *Saeed Al-Tala*[8], *Fowzan S Alkuraya*[7,9], *Alberta Leon*[10], *R.L.E. van Loon*[11], *Alessandra Ferlini*[12], *Mariabeatrice Sanchini*[12], *Stefania Bigoni*[12], *Mitchell O'Connell*[13], *Bogdan Polevoda*[13], *Kamel Awayda*[13], *Stéphane Bézieau*[1,2], *Benjamin Cogné*[1,2]

[1]*Service de Génétique Médicale, CHU de Nantes, Nantes, France,* [2]*Université de Nantes, CNRS, INSERM, l'institut du thorax, Nantes, France,* [3]*Department of Neurology, Children's Health Ireland at Temple Street, Dublin, Ireland,* [4]*Exeter Genomics Laboratory, Royal Devon and Exeter NHS Foundation Trust, Exeter, United Kingdom,* [5]*Institute of Biomedical and Clinical Science, University of Exeter Medical School, Exeter, United Kingdom,* [6]*Section of Medical Genetics, Children's Hospital, King Fahad Medical City, Riyadh, Saudi Arabia,* [7]*Department of Translational Genomics, Center for Genomics Medicine, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia,* [8]*Pediatrics Department, Armed Forces Hospital, Khamis Mushait, Saudi Arabia,* [9]*College of Medicine, Alfaisal University, Riyadh, Saudi Arabia,* [10]*Research & Innovation (R&I Genetics) Srl, Genetic Laboratory, Padua, Italy,* [11]*Department of Genetics, University of Utrecht, University Medical Center Utrecht, Utrecht, Netherlands,* [12]*Medical Genetics Unit, Department of Medical Sciences, University of Ferrara, Ferrara, Italy,* [13]*Department of Biochemistry and Biophysics, Center for RNA Biology, University of Rochester, Rochester, NY, USA.*

Introduction: tRNA-modifications such as methylation via tRNA-methyltransferase (TRMT1) or NOP2/Sun RNA Methyltransferase 2 (NSUN2) have been implicated in autosomal recessive intellectual developmental disorder (ARIDD). THUMPD1 carries a thiouridine synthases, methylases and pseudouridine synthases (THUMP) RNA-binding domain involved in tRNA acetylation. Recently, this gene was proposed as a candidate gene for ARIDD. Here we present a series of 8 patients showing syndromic intellectual disability with homozygous or compound heterozygous THUMPD1 variants.

Materials and Methods: Whole exome sequencing (WES) was performed on blood-derived DNA samples following each center's Next Generation Sequencing (NGS) pipeline. Exome data were interpreted in agreement with local practices. Phenotypes were reported by each attending physician.

Results: We describe 8 patients from 5 families, most of them born from consanguineous parents, carrying rare presumptive loss of function variants in THUMPD1. Common phenotypic findings included: microcephaly, global developmental delay, speech delay, moderate to severe intellectual deficiency, behavioral abnormalities such as angry outbursts, facial dysmorphism and ophthalmological abnormalities.

Conclusions: Given the genotypic and phenotypic similarities in our case series, we propose that recessive variants in THUMPD1 should be considered causal of syndromic intellectual disability. We describe a new neurodevelopmental syndrome with intellectual disability, developmental delay, behavioral abnormalities and facial dysmorphism.

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P08.038.D Efficient application of next-generation sequencing for the diagnosis of neurodevelopmental diseases

Irene Madrigal, *Maria Isabel Alvarez-Mora, Aurora Sanchez, Laia Rodriguez-Revenga*

Biochemistry and Molecular Genetics, Barcelona, Spain.

Introduction: Neurodevelopmental diseases affect 2%-3% of the general population, and have highly clinical and genetic heterogeneity, which complicates the genetic diagnosis. In fact the genetic defect remains unknown in around 40% of patients. The application of next-generation sequencing is changing the nature of biomedical diagnosis. This technology has quickly become the method of choice for searching for pathogenic mutations in rare genetic diseases.

Material and Methods: In order to identify variants underlying disease phenotypes, we applied whole-exome or genome sequencing to 59 families with one or several members affected with intellectual disability.

Results: Identification of disease-causing mutations was achieved in 42% of studied families (25/59) who could receive a genetic diagnosis and counselling. All identified genes are related to ID although the 80% of the variants had not been previously described. Regarding the inheritance, 40% were autosomic dominant, 36% were X-linked, 20% were autosomic recessive and 4% were imprinting mutations.

Conclusions: The accessibility to next-generation sequencing allows clinicians to save much time and cost in identifying the aetiology of rare diseases. The presented cases are excellent examples that demonstrate the efficacy of next-generation sequencing in rare disease diagnosis. Acknowledgements: This study was supported by the Instituto de Salud Carlos III [PI12/00879], co-financed by Fondo Europeo de Desarrollo Regional (FEDER) "una manera de hacer Europa", AGAUR from the Autonomous Catalan Government [2017 SGR1134] and Fundación Alicia Koplowitz (AKOPLOWITZ18_001). The 'CIBER de Enfermedades Raras' is an initiative of the ISCIII. We want to thank the "CERCA Programme" from the Autonomous Catalan Government.

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P08.039.A Dominant variants in *ITSN1* cause neurodevelopmental disorder spectrum

Ange-Line Bruel[1,2], *Antonio Vitobello*[1,2], *Isabelle Thiffault*[3], *Linda Manwaring*[4], *Marcia Willing*[4], *Pankaj B. Agrawal*[5], *Allan Bayat*[6], *Thomas M. Kitzler*[7,8], *Catherine A. Browntein*[9], *Casie A. Genetti*[10], *Joseph Gonzales-Heydrich*[11], *Parul Jayakar*[12], *Jacob W. Zyskind*[13], *Zehua Zhu*[13], *Clemence Vachet*[14], *Gena R. Wilson*[15], *Brianna Pruniski*[15], *Anne-Marie Goyette*[16], *Yannis Duffourd*[1,2], *Christophe Philippe*[1,2,17], *Christel Thauvin-Robinet*[1,17,2], *Laurence Faivre*[2,17,1]

[1]*UMR1231 INSERM, GAD, Dijon, France,* [2]*Unité Fonctionnelle Innovation en Diagnostic génomique des maladies rares, FHU-TRANSLAD, CHU Dijon Bourgogne, Dijon, France,* [3]*Center for Pediatric Genomic Medicine, Children's Mercy Hospital, Kansas City, KS, USA,* [4]*Department of Pediatrics, Division of Genetics and Genomic Medicine, Washington University School of Medicine, St Louis, MO, USA,* [5]*Divisions of Newborn Medicine, Genetics and Genomics, The Manton Center for Orphan Disease Research, Boston Children's Hospital, Boston, Boston, MA, USA,* [6]*Department of Genetics and Precision Medicine, Danish Epilepsy Centre, Dianalund, Denmark,* [7]*Research Institute, McGill University Health Centre, Montreal, Quebec; Division of Medical Genetics, Department of Medicine, McGill University Health Centre, Montreal, QC, Canada,* [8]*Department of Human Genetics, McGill University, Montreal, QC, Canada,* [9]*Divisions of Newborn Medicine, Genetics and Genomics, The Manton Center for Orphan Disease Research, Boston Children's Hospital, Boston, MA, USA,* [10]*Divisions of Genetics and Genomics, Department of Pediatrics, The Manton Center for Orphan Disease Research, Boston Children's Hospital, Boston, MA, USA,* [11]*Department of Psychiatry, Boston Children's Hospital, Manton Center for Orphan Disease Research, Division of Genetics and Genomics, Boston Children's Hospital, Department of Pediatrics, Harvard Medical School, Boston, MA, USA,* [12]*Division of Genetics and Metabolism, Nicklaus Children's Hospital, Miami, FL, USA,* [13]*GeneDX, Gaitherburg, MD, USA,* [14]*Service de néphrologie pédiatrique, Centre Hospitalier Régional Universitaire Besançon, Besançon, France,* [15]*Division of Genetics and Metabolism, Phoenix Children's Medical Group, Phoenix, AZ, USA,* [16]*FRCPC, Developmental Pediatrician, Montreal Children's Hospital, McGill University Health Center, Montreal, QC, Canada,* [17]*Fédération Hospitalo-Universitaire Médecine Translationnelle et Anomalies du Développement (TRANSLAD), Centre Hospitalier Universitaire Dijon, Dijon, France.*

We described *de novo* and inherited missense and truncating variants in *ITSN1* as a novel cause of neurodevelopmental disorder spectrum in a total of 8 unrelated patients. A review of the literature identified four additional patients from large meta-analysis studies. ITSN1 plays an important role in brain development including including development of dendritic spines, cortical midline connectivity, synaptic vesicle recycling, neuronal migration, synaptic plasticity and more recently in learning and memory. We evidenced that missense *ITSN1* variants (3/12 variants) without splicing defect predicted are spatially clustered in C-terminal in an important regional missense constraint. Missense variants are predicted probably damaging by PolyPhen-2. GnomAD database reported the intolerance of *ITSN1* gene to support missense variants (misZ-score = 3.61). Variants causing premature codon stop (9/12 variants) are located in the first half of the protein. *ITSN1* showed a high intolerance to inactivation reported by GnomAD database with an associated pLI (probability of loss-of-function intolerance) score of 1. Neurological disorders were diagnosed in all patients and included intellectual disability or global developmental delay (8/8) and autism spectrum disorders (10/11). Seizures free was noted in 2/8 patients. All patients showed speech delay and/or language impairment (8/8), three displayed a regression. Mainly patients presented severe behavioural troubles and/or severe psychiatric disorders. Minor and inconstant dysmorphic features were observed. We suggest *ITSN1* gene is involved in development of an autism spectrum disorder with variable additional neurodevelopmental deficiency, thus confirming the hypothesis that *ITSN1* is important for brain development.

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P08.040.B Expanding the mutational landscape and clinical phenotype of the *YIF1B* related brain disorder

Eva Medico Salsench[1], *Reza Maroofian*[2], *Kristina Lanko*[1], *Belén Pérez Gonzalez*[3], *Obdulia Sanchez-Lijarcio*[3], *Salvador Ibáñez-Mico*[4], *Antonina Wojcik*[5], *Marcello Vargas*[5], *Audrey Schroeder*[6], *Chin-To Fong*[6], *Amber Begtrup*[7], *Ibrahim H Kaya*[8], *Mohammad AlMuhaizea*[8,9], *Dilek Colak*[10], *Henry Houlden*[2], *Robert-Jan Galjaard*[1], *Namik Kaya*[10], *Tahsin Stefan Barakat*[1]

[1]*Erasmus MC University Medical Center, Rotterdam, Netherlands,* [2]*UCL Queen Square Institute of Neurology, London, United Kingdom,* [3]*Centro de Biología Molecular, Universidad Autonoma de Madrid, Madrid, Spain,* [4]*Arrixaca Universitary Hospital, Murcia, Spain,* [5]*Gillette Children's Specialty Healthcare, St. Paul, MN, USA,* [6]*University of Rochester Medical Center, Rochester, NY, USA,* [7]*GeneDx, Gaithersburg, MD, USA,* [8]*AlFaisal University, Riyadh, Saudi Arabia,* [9]*King Faisal Specialist Hospital and Research, Riyadh, Saudi Arabia,* [10]*King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia.*

Intracellular proteins involved in mediating vesicular trafficking in eukaryotic cells have been implicated in brain disorders, showing the relevance of the process for neuronal development in human. YIF1B is an essential protein involved in the anterograde trafficking from the endoplasmic reticulum to the cell membrane, and in Golgi apparatus architecture. We recently described a neurodevelopmental disorder caused by recessive variants in *YIF1B*, which has now been recognized by OMIM as Kaya-Barakat-Masson syndrome (KABAMAS, OMIM# 619125). So far, our study (Almuhaizea *et al*.) and that of Diaz *et al*. reported 16 affected individuals from 11 independent families. These individuals presented with a progressive encephalopathy with various degrees of movement disorders, microcephaly, and epilepsy. In all but one family, bi-allelic protein truncating variants were identified in *YIF1B*, with only a single bi-allelic missense mutation assumed to be causative. Here, we describe 6 additional individuals from 6 families harboring protein altering variants in *YIF1B*, 4 of which are homozygous or compound heterozygous missense variants. Interestingly, all *YIF1B* missense variants encountered localized in, or close to, the transmembrane domains, which were previously shown to be essential for YIF1B function. To investigate the function of these missense variants, we performed site-directed mutagenesis followed by expression and interaction studies, providing functional evidence from in vitro studies that these missense variants impact on YIF1B function. In addition, we compare the clinical phenotype between all currently known YIF1B cases to further delineate the mutational

landscape and the clinical phenotype associated with this new disease entity.

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P08.041.C Frameshift variant in *SETD5* in a patient presenting a KBG syndrome

Angela Teresa Abad Perez[1], *Felix Boschann*[1], *Birgit Jödicke*[2], *Denise Horn*[1]

[1]*Institute of Medical Genetics and Human Genetics, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany, Berlin, Germany,* [2]*Center for Chronically Sick Children, Department of Paediatric Endocrinology and Diabetes, Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany.*

KBG syndrome (KBGS) is a developmental disorder characterized by distinctive craniofacial features, macrodontia of the upper central incisors, skeletal anomalies, short stature and neurologic involvement that may include seizures and intellectual disability. It is caused by sequence variants in *ANKRD11* and the 16q24.3 deletion, which includes *ANKRD11*. For a small number of affected individuals, a causative *ANKRD11* variant cannot be detected. Here, we describe a 16-year-old female patient with macrodontia of the upper central incisors, short stature and developmental delay. Hand radiographs showed a shortening of the 5th metacarpal. At age 7, she was clinically diagnosed with KBGS. Analysis of *ANKRD11* revealed no mutations or deletions of the gene. Exome sequencing revealed a heterozygous *de novo* frameshift-mutation in the *SETD5* gene. Haploinsufficiency of *SETD5* causes intellectual disability with minor facial dysmorphism and skeletal anomalies (mental retardation, autosomal dominant 23, MRD23) and is believed to be causative of the core phenotype of the microdeletion 3p25.3 syndrome. Loss-of-function variants in *SETD5* were recently reported in three patients with an initial KBGS clinical diagnosis. Reevaluation of previously reported cases revealed a significant clinical overlap between KBG, MRD23 and microdeletion 3p25.3 syndromes. (Crippa et al., 2019) *ANKRD11* and *SETD5* have been identified as chromatin regulators involved in gene expression. In mouse embryonic stem cells, both proteins have been shown to physically interact at the molecular level. This report provides further evidence that *SETD5* mutations can cause KBGS phenotype. Targeted analysis of *SETD5* should be considered in unsolved KGBS patients.

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P08.042.D KCNB1 frameshift variant caused inherited intellectual disability, developmental delay and seizure

Eyyup Uctepe[1], *Fatma Nisa Esen*[2], *Sait Tümer*[2], *Ahmet Yesilyurt*[2]

[1]*Acıbadem Labmed/Ankara Tissue Typing Laboratory, Ankara, Turkey,* [2]*Acibadem Labgen Genetic Diagnosis Center, İstanbul, Turkey.*

Introduction: Potassium voltage-gated channel subfamily B member 1 (*KCNB1*) encodes Kv2.1 potassium channel. *KCNB1* mutations are known to cause global developmental delay, behavioral disorders, and various epilepsies. Most variants occur de novo and rarely inherited. Here, we report a 14-year-old male patient who was admitted to our clinic with seizures, developmental delay history, and mild intellectual disability. Brain magnetic resonance image (MRI) was normal and electroencephalogram (EEG) showed spike and sharp-wave complexes emerging in the left hemisphere parietooccipital areas which paroxysmally generalized.

Materials and Methods: We performed whole exome sequence analysis (WES) in the proband.

Results: WES identified a heterozygous frameshift mutation c.522delA in exon 1 of *KCNB1* (NM_004975.4) predicting a premature stop codon p.Lys174Asnfs*20 in the proband. Sanger sequencing confirmed the heterozygous c.522delA mutation in the proband and his mother who had also epilepsy and mild intellectual disability. His 45 years old mother had used antiepileptic drugs for 9 years after a seizure episode at 12 years old. Also, the mother's uncle's son is nonverbal and has developmental delay and epilepsy.

Conclusions: Our study shows that frameshift mutation in *KCNB1* gene can cause intrafamilial phenotypic variability and relatively milder clinical findings in these patients.

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P08.043.A GenIDA, an international participatory database to get an insight into the natural history and co-morbidities of genetic forms of neurodevelopmental disorders

Jean-Louis Mandel[1,2], *Pauline Burger*[1,2], *Axelle Strehle*[1,2], *Florent Colin*[1,2], *Timothée Mazzucotelli*[1], *Nicole Collot*[1], *Amélie Piton*[1,2,3], *Pierre Parrend*[2,4], *Laurence Faivre*[5,6], *David Geneviève*[7], *Valentin Ruault*[7], *Thomas Smol*[8], *Roseline Caumes*[9], *Joost Kummeling*[10], *Charlotte Ockeloen*[10], *Tjitske Kleefstra*[10], *David Koolen*[10]

[1]*IGBMC, Illkirch, France,* [2]*Université de Strasbourg, Strasbourg, France,* [3]*Unité de Génétique Moléculaire, Hôpitaux Universitaires de Strasbourg, Strasbourg, France,* [4]*ECAM Strasbourg-Europe, Schiltigheim, France,* [5]*Equipe Génétique des Anomalies du Développement, Université de Bourgogne-Franche Comté, Dijon, France,* [6]*Centre de Génétique et Centre de Référence Anomalies du Développement et Syndromes Malformatifs, FHU TRANSLAD, Hôpital d'Enfants, CHU Dijon et Université de Bourgogne, Dijon, France,* [7]*Université de Montpellier, Centre Hospitalier Universitaire Montpellier, CLAD Sud Languedoc-Roussillon, INSERM, Montpellier, France,* [8]*CHU Lille, Institut de Génétique Médicale, RADEME, Lille, France,* [9]*CHU Lille, Clinique de Génétique Guy Fontaine, Lille, France,* [10]*Department of Human Genetics, Donders Institute for Brain, Cognition and Behavior, Radboud University Medical Center, Nijmegen, Netherlands.*

GenIDA is an international online project initiated with the aim of better characterising the clinical manifestations and natural histories of genetic forms of intellectual disability with or without autism or epilepsy. Clinical information reported and updated by the proband's family using a structured questionnaire, is analysed to identify new medically significant information for families and professionals concerned by a given condition. The current questionnaire consists of 41 multiple-choice questions exploring physical parameters, cognitive and behavioural aspects, presence or absence of neurological manifestations or problems affecting major physiological functions (cardiac, respiratory, renal…). Five open questions explore the families' perception of manifestations which most affect health and quality of life of their relative, events of adverse reactions to treatments, etc. Currently, the questionnaire is available in 7 languages and has been filled for 1080 patients, the main cohorts being Koolen-de Vries/KdVS (n = 210),

Kleefstra (147) and KBG (40) syndromes. This allowed identification of respiratory problems in KdVS not reported previously, and of differences in behavioural manifestations between the 3 syndromes. Others cohorts have experienced significant growth over the last year (MED13L: 36, DDX3X: 32; POGZ:10). Comparing sleep and epilepsy aspects for these 6 conditions reveal major differences. For instance, for POGZ, sleep disorders appear much more frequent than epilepsy. For KdVS, epilepsy that affects almost 50% of patients is of much greater concern than sleep problems.

Conclusion: The data validate the interest of our participatory approach: through their direct involvement, families can reveal aspects of the pathology that have been underestimated until now.

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P08.045.C Deletions in *MACROD2* gene and Autism Spectrum Disorders

Fernando Macho Carballido, *Javier Torres Hernández, Maria Luisa Quintanilla Mata, María Esther Simarro Rueda, Laura Navarro Casado, Elia López Ballesteros*

Hospital General Universitario de Albacete, Albacete, Spain.

Introduction: Autism Spectrum Disorders (ASD),which are defined as a chronic neurological disorder with a strong genetic basis, manifests at an early age with a variety of symptoms related to social interaction, communication and lack of flexibility in reasoning and behavior. *MACROD2* is a gene involved in DNA repair, cell signaling, gene transcription, and chromatin remodeling. It is highly expressed in the ventricular zone of the brain during embryonic development. Deletions of 20p12.1 involving *MACROD2* have been associated with ASD according to several studies that preliminarily linked this gene to ASD. CASE REPORT First, in 2012, in a 12-year-old patient diagnosed with ASD, a 400 K array-CGH was made, resulting in a deletion of 86 Kb in 20p12.1 (chr20: 14147320_14234229). This CNV was classified as of uncertain clinical significance and was not considered as the cause of the disorder. Recently, in another 3-year-old patient with ASD, a genetic study was carried out using a 180K CGH-array, finding a 0.265 Mb deletion in 20p12.1 (chr20: 14776880_15041538) in the *MACROD2* gene. In both cases, the deletions were inherited from healthy mothers, so these alterations could have incomplete penetrance and high phenotypic variability.

Discussion: Based on these cases and others previously reported [Lombardo et al., 2019; Frye et al., 2016], where deletions in 20p12.1 are found in non-syndromic boys with ASD, it would be necessary to review old cases in which no cause-effect was found between the deletion and the disorder.

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P08.047.D Functional analysis of *TLK2* variants and proximal interactome support a pathogenic mechanism based on impaired kinase activity causing alteration of chromatin stability pathways

Lisa Pavinato[1,2], *Marina Villamor-Payà*[3], *Maria Sanchiz-Calvo*[3], *Cristina Andreoli*[4], *Marina Gay*[3], *Marta Vilasec*[3], *Gianluca Arauz-Garofalo*[3], *Andrea Ciolfi*[5], *Alessandro Bruselles*[6], *Tommaso Pippucci*[7], *Valentina Prota*[4], *Diana Carli*[8], *Elisa Giorgio*[1,9], *Francesca Clementina Radio*[5], *Vincenzo Antona*[10], *Mario Giuffrè*[10], *Kara Ranguin*[11], *Cindy Colson*[11], *Silvia De Rubeis*[12,13,14], *Paola Dimartino*[15], *Joseph Buxbaum*[12,13,14], *Giovanni Battista Ferrero*[16,17], *Marco Tartaglia*[5], *Simone Martinelli*[6], *Travis H. Stracker*[3,18], *Alfredo Brusco*[1,19]

[1]*Department of Medical Sciences, University of Turin, Turin, Italy,* [2]*Institute of Human Genetics and Center for Molecular Medicine Cologne, University of Cologne, Köln, Germany,* [3]*The Barcelona Institute of Science and Technology, Institute for Research in Biomedicine, Barcelona, Spain,* [4]*Department of Environment and Health, Istituto Superiore di Sanità, Rome, Italy,* [5]*Genetics and Rare Diseases Research Division, Ospedale Pediatrico Bambino Gesù IRCCS, Rome, Italy,* [6]*Department of Oncology and Molecular Medicine, Istituto Superiore di Sanità, Rome, Italy,* [7]*Medical Genetics Unity, Sant'Orsola-Malpighi University Hospital, Bologna, Italy,* [8]*Department of Pediatrics and Public Health and Pediatric Sciences, University of Turin, Turin, Italy,* [9]*Department of Molecular Medicine, University of Pavia, Pavia, Italy,* [10]*Department of Sciences for Health Promotion and Mother and Child Care "G. D'Alessandro", University of Palermo, Palermo, Italy,* [11]*Department of Genetics, Reference center for Rare Diseases and Developmental Anomalies, Caen, France,* [12]*Seaver Autism Center for Research and Treatment, Icahn School of Medicine at Mount Sinai, New York City, NY, USA,* [13]*Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York City, NY, USA,* [14]*The Mindich Child Health and Development Institute, Icahn School of Medicine at Mount Sinai, New York City, NY, USA,* [15]*Department of Medical and Surgical Sciences, Bologna, Italy,* [16]*Department of Pediatrics and Public Health and Pediatric Sciences, Turin, Italy,* [17]*Clinical and Biological Sciences Department, University of Turin, Turin, Italy,* [18]*Radiation Oncology Branch, National Cancer Institute, Bethesda, MD, USA,* [19]*Unit of Medical Genetics, "Città della Salute e della Scienza" University Hospital, Turin, Italy.*

The Tousled-Like Kinases 1 and 2 (TLK1 and TLK2) are involved in many fundamental processes, including DNA replication, cell cycle checkpoint recovery and chromatin remodeling. Variants in *TLK2* were associated with "Mental Retardation Autosomal Dominant 57" (MRD57), a neurodevelopmental disorder characterized by a highly variable phenotype, including intellectual disability, behavioral abnormalities, facial dysmorphisms, microcephaly, epilepsy, and skeletal anomalies. By whole exome sequencing and array-CGH analysis, we identified three unrelated MRD57 cases. Two were sporadic and caused by a missense change (c.1652A>G; p.(Asp551Gly)) and a 39-kb deletion encompassing *TLK2*, and one was familial with three affected siblings who inherited a nonsense change from an affected mother (c.1423G>T; p.(Glu475Ter)). Using spatial proteomics (BioID) and single-cell gel electrophoresis, we investigated the proximity interaction landscape of *TLK2* and analyzed the effects of p.(Asp551Gly) and a previously reported missense variant (c.1850C>T; p.(Ser617Leu)) on TLK2 interactions, localization and activity. Proximal interactions between TLK2 and other factors implicated in neurological disorders, including CHD7, CHD8, BRD4, NACC1, were identified. Notably, most of these interactions were altered by the analyzed missense variants, as well as TLK2 kinase activity and localization. Furthermore, we demonstrated a more relaxed chromatin state in lymphoblastoid cells harboring the p.(Asp551Gly) variant compared to control cells, conferring susceptibility to DNA damage. Overall, our study identified novel patients carrying pathogenic variants confirming and further expanding MRD57-related phenotype. By means of

interactome and functional analysis, we have molecularly characterized two missense variants, providing new insights on the pathomechanistic consequences of *TLK2* mutations on intellectual disability and neurodevelopmental disorders.

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P08.048.B A *MT-TL1* variant identified by whole exome sequencing in an individual with intellectual disability, epilepsy and spastic tetraparesis

Elke de Boer[1], *Charlotte W. Ockeloen*[2], *Leslie Matalonga*[3], *Rita Horvath*[4], *Solve-RD SNV-indel working group, Richard J. Rodenburg*[5], *Marieke J. H. Coenen*[6], *Mirian Janssen*[7], *Dylan Henssen*[8], *Christian Gilissen*[9], *Wouter Steyaert*[9], *Ida Paramonov*[3], *Solve-RD-DITF-ITHACA, Aurélien Trimouille*[10], *Tjitske Kleefstra*[1], *Alain Verloes*[11], *Lisenka E. L. M. Vissers*[1]

[1]*Department of Human Genetics, Donders Institute for Brain, Cognition and Behaviour, Radboudumc, Nijmegen, Netherlands,* [2]*Department of Human Genetics, Radboudumc, Nijmegen, Netherlands,* [3]*CNAG-CRG, Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Barcelona, Spain,* [4]*Department of Clinical Neurosciences, University of Cambridge, John Van Geest Cambridge Centre for Brain Repair, Cambridge, United Kingdom,* [5]*Department of Laboratory Medicine, Translational Metabolic Laboratory, Radboudumc, Nijmegen, Netherlands,* [6]*Department of Human Genetics, Radboud Institute for Health Sciences, Radboudumc, Nijmegen, Netherlands,* [7]*Department of Internal Medicine, Radboudumc, Nijmegen, Netherlands,* [8]*Department of Medical Imaging, Radboudumc, Nijmegen, Netherlands,* [9]*Department of Human Genetics, Radboud Institute for Molecular Life Sciences, Radboudumc, Nijmegen, Netherlands,* [10]*Laboratoire de Génétique Moléculaire, Service de Génétique Médicale, CHU Bordeaux – Hôpital Pellegrin, Bordeaux Cedex, France,* [11]*Département de Génétique, APHP Robert DEBRE University Hospital and INSERM U1141, Paris, France.*

The genetic etiology of intellectual disability remains elusive in almost half of all affected individuals. Within the Solve-RD consortium, systematic re-analysis of whole-exome sequencing (WES) data from unresolved cases with intellectual disability (n = 1,472 probands) was performed. This re-analysis included variant calling of mitochondrial DNA (mtDNA) variants, although mtDNA is not specifically targeted in WES. We identified a functionally relevant mtDNA variant in *MT-TL1* (NC_012920.1:m.3291T>C; NC_012920.1:n.62T>C), at a heteroplasmy level of 22% in whole blood, in a 23-year-old male with severe intellectual disability, epilepsy, episodic headaches with emesis, spastic tetraparesis, brain abnormalities and feeding difficulties. Targeted validation in blood and urine supported pathogenicity, with heteroplasmy levels of 23% and 58% in index, and 4% and 17% in mother, respectively. Interestingly, not all phenotypic features observed in the index have been previously linked to this *MT-TL1* variant, suggesting either broadening of the m.3291T>C-associated phenotype, or presence of a co-occurring disorder. Hence, our case highlights the importance of underappreciated mtDNA variants identifiable from WES data, especially for cases with atypical mitochondrial phenotypes and their relatives in the maternal line.

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P08.049.C A prospective study highlighting the increasing role of multiple molecular diagnoses in the field of rare diseases

Caroline Racine[1,2,3], *Camille Engel*[2], *Frédéric Tran Mau-Them*[1,2], *Ange-Line Bruel*[1,2], *Antonio Vitobello*[1,2], *Arthur Sorlin*[1,2,3], *Anne-Sophie Denommé-Pichon*[1,2,3], *Hana Safraou*[1,2,3], *Sophie Nambot*[1,3], *Julian Delanne*[1,3], *Aurore Garde*[1,3], *Marie Bournez*[3], *Sébastien Moutton*[1,3], *Julien Thevenon*[3], *Daphnée Lehalle*[3], *Nada Houcinat*[3], *Nolwenn Jean-Marçais*[3], *Marjolaine Willems*[4], *Alain Verloes*[5], *Fanny Laffargue*[6], *Lucile Pinson*[4], *James Lespinasse*[7], *Elodie Lacaze*[8], *David Geneviève*[4], *Olivier Patat*[9], *Laetitia Lambert*[10], *Marion Gérard-Blanluet*[11], *Charlotte Benigni*[10], *Orphanomix Physician's Group, Philippine Garret*[1,2], *Christophe Philippe*[1,2], *Yannis Duffourd*[1,2], *Laurence Faivre*[1,2,3], *Christel Thauvin-Robinet*[1,2,12]

[1]*Inserm UMR 1231 GAD, Genetics of Developmental disorders, Université de Bourgogne-Franche Comté, FHU TRANSLAD, Dijon, France,* [2]*Unité Fonctionnelle "Innovation diagnostique dans les maladies rares" laboratoire de génétique chromosomique et moléculaire, Plateau Technique de Biologie, CHU Dijon, Dijon, France,* [3]*Centre de Référence Maladies Rares "Anomalies du Développement et syndromes malformatifs", FHU-TRANSLAD, CHU Dijon, Dijon, France,* [4]*Département de Génétique Médicale, Maladies rares et Médecine Personnalisée, CHU de Montpellier, Montpellier, France,* [5]*Département de Génétique, Assistance Publique des Hôpitaux de Paris (AP-HP) Hôpital Robert Debré, Paris, France,* [6]*Service de Génétique Médicale, Centre Hospitalier Universitaire Estaing, Clermont-Ferrand, France,* [7]*Laboratoire de Génétique Chromosomique, CH Général, Chambéry, France,* [8]*Département de génétique, CH du Havre, Le Havre, France,* [9]*Service de Génétique Médicale, Hôpital Purpan, Centre Hospitalier Universitaire, Toulouse, France,* [10]*Service de Génétique Clinique, CHU de Nancy, Nancy, France,* [11]*Service de Génétique, CHU de Caen, Caen, France,* [12]*Centre de Référence Maladies Rares "Déficiences Intellectuelles de causes rares", FHU-TRANSLAD, CHU Dijon, Dijon, France.*

Introduction: The utility of next-generation sequencing (NGS) technologies in the field of rare diseases has already been proven, improving the molecular diagnosis rate. Among those diagnoses, multiple molecular diagnoses have been reported in the literature, ranging from 3.2% to 7% of positive diagnoses. We here analyze their characteristics in a study on exome sequencing (ES) data.

Materials and Methods: ES data were obtained from a cohort of 1859 unrelated patients, referred to our diagnostic laboratory for the etiological work-up of intellectual disability and/or congenital abnormalities. Data were analyzed prospectively from January 2017 to December 2020.

Results: A molecular diagnosis was raised for 615/1859 patients (33.1%). Among them, 21 (3.4%) had two pathogenic variants and 20 additional patients had one pathogenic and one variant of unknown significance (VUS) with a high probability of being reclassified as likely pathogenic in the future, raising the rate to 6.7%. Causal copy-number variants were found in 9/21 patients. Two patients displayed a second variant involving a distinct disease. Both variants contributed to a complex phenotype for 10 patients. For the remaining 9, the second variant modulated the phenotype. When parental samples were

available, both variants were found *de novo* for 1/16 patient, and one variant *de novo* for 9/16 patients.

Conclusions: Our results are consistent with the literature since multiple molecular diagnoses occurred in 3.4% of positive cases – 6.7% if VUS are considered. It suggests their increasing role in rare diseases and the great value of taking them into consideration within a laboratory diagnostic routine.

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P08.050.D MYT1L-associated neurodevelopmental disorder: a clinical and molecular description of 37 new cases and literature review

Juliette Coursimault[1], *Anne-Marie Guerrot*[1], *Michelle Morrow*[2], *Bert Callewaert*[3], *Sarah Vergult*[3], *Laurence Faivre*[4], *Frédéric Tran Mau-Them*[4], *Ange-Line Bruel*[4], *Estelle Colin*[5], *Marine Tessarech*[6], *Mathilde Nizon*[7], *Benjamin Cogne*[7], *Bertrand Isidor*[7], *Cyril Mignot*[8], *Diane Doummar*[9], *Boris Keren*[8], *Stéphanie Valence*[9], *Delphine Héron*[8], *Françoise Devillard*[10], *Charles Coutton*[11], *Marlène Rio*[12], *Karine Poirier*[12], *Elise Schaefer*[13], *Bénédicte Gérard*[14], *Gwenaël Le Guyader*[15], *Frédéric Bilan*[15], *Wendy Chung*[16], *Rebecca Hernan*[16], *Austin Larson*[17], *Kelly Nori*[17], *Sarah Stewart*[17], *James Wheless*[18], *Salima El Chehadeh*[13], *Beth Pletcher*[19], *Christina Kresge*[19], *Margaret Helm*[20], *Laurence Colleaux*[12], *Anne-Sophie Alaix*[12], *Jeanne Amiel*[12], *Sophie Rondeau*[12], *Roseline Caumes*[21], *Thomas Smol*[22], *Sabine Sigaudy*[23], *Alexandra Afenjar*[8], *Christine Coubes*[24], *Christine Poitou*[25], *Thierry Frébourg*[1], *Pascale Saugier-Veber*[1], *Gaël Nicolas*[1], *François Lecoquierre*[1]

[1]*Normandie Univ, UNIROUEN, Inserm U1245, CHU Rouen, Department of Genetics and reference center for developmental disorders, FHU G4 Génomique, F-76000, Rouen, France,* [2]*GeneDX, 207 Perry Parkway Gaithersburg, MD 20877, Gaithersburg, MD, USA,* [3]*Center for Medical Genetics, Ghent University Hospital, Department of Biomolecular Medicine, Ghent University, Ghent, Belgium,* [4]*Service de Génétique des Anomalies du Développement, CHU Dijon, Dijon, France,* [5]*Department of Biochemistry and Genetics, Angers University Hospital, Angers, France UMR CNRS 6015-INSERM 1083 and PREMMI, Mitovasc Institute, Angers, France,* [6]*Department of Biochemistry and Genetics,Angers University Hospital, Angers, France UMR CNRS 6015-INSERM 1083 and PREMMI, Mitovasc Institute, Angers, France,* [7]*CHU Nantes, Service de Génétique Médicale, Nantes, France,* [8]*APHP. Sorbonne Université, Département de Génétique, Groupe Hospitalier Pitié-Salpêtrière-Hôpital Trousseau, Centre de Référence Déficiences Intellectuelles de Causes Rares, Paris, France,* [9]*APHP.Sorbonne Université, Service de Neuropédiatrie, Hôpital Trousseau, Paris, France,* [10]*Service de génétique et procréation CHU Grenoble Alpes, Grenoble, France,* [11]*Genetic Epigenetic and Therapies of Infertility, Institute for Advanced Biosciences, Institut National de la Santé et de la Recherche Médicale U1209, Centre National de la Recherche Scientifique UMR 5309, Université Grenoble Alpes, Grenoble, France,* [12]*Department of Genetics, IHU Necker-Enfants Malades, University Paris Descartes, Paris, France,* [13]*Service de génétique médicale, Hôpitaux Universitaires de Strasbourg, Institut de Génétique Médicale d'Alsace, Strasbourg, France,* [14]*Laboratoire de Diagnostic Génétique, Hôpitaux Universitaires de Strasbourg, Strasbourg, France,* [15]*Centre Hospitalier Universitaire de Poitiers, Service de Génétique, BP577, 86021, Poitiers, France,* [16]*Columbia University Irving Medical Center, New York, NY, USA,* [17]*University of Colorado, School of Medicine and Children's Hospital, Aurora, CO, USA,* [18]*Division of Pediatric Neurology, University of Tennessee, Health Science Center, Memphis, TN, USA,* [19]*Division of Clinical Genetics, Rutgers New Jersey Medical School, Newark, NJ, USA,* [20]*Maine Medical Partners, Pediatric Specialty Care Genetics, Portland, ME, USA,* [21]*Institut de Génétique médicale, Clinique Guy de Fontaine, Jeanne de Flandre, Lille, France,* [22]*Pôle Biologie Pathologie Génétique, Centre de Biologie Pathologie Pierre-Marie Degand, Lille, France,* [23]*Département de Génétique Médicale, Hôpital Timone Enfant, Marseille, France,* [24]*Département de Génétique Médicale, Maladies Rares et Médecine Personnalisée, CHU Montpellier, Montpellier, France,* [25]*Service de Nutrition, Hôpital de la Pitié Salpêtrière - AP-HP, Paris, France.*

Introduction: Pathogenic variants of the myelin transcription factor-1 like (*MYT1L)* gene cause a syndromic neurodevelopmental disorder and include missense, premature termination codon (PTC) variants and 2p25.3 microdeletions. Despite a strong enrichment in *de novo* mutations in developmental disorders or autism trio studies, the clinical characterization and phenotype-genotype correlations are scarce and only 21 patients with missense or PTC variants have been reported so far.

Materials and Methods: We collected clinical and genetic information of 37 new patients with (likely) pathogenic *MYT1L* variants through datasharing resources and collaborations and performed a comprehensive meta-analysis with already published data (total = 58 patients).

Results: We first confirmed that the main phenotypic features of the *MYT1L*-related disorder are developmental delay (95%), intellectual disability (ID, 68%), overweight or obesity (59%), behavioral disorders (100%) and epilepsy (22%). In addition, 32% of the patients presented learning disorders without ID and 19% presented in infancy feeding difficulties, which were not reported before. We further describe the inconstant dysmorphic features (69%) and present the weight evolution of 21 patients. We show that patients harboring highly clustered missense variants within the 2nd and 3rd zinc finger domains are not clinically distinguishable from patients with truncating variants. We report the first *de novo* missense variants outside the 2nd and 3rd zinc finger domains, which nevertheless remain the target domains for most pathogenic missense variants.

Conclusion: We provide an updated description of clinical and genetic data of the *MYT1L*-associated neurodevelopmental disorder, hence improving diagnosis and clinical management of these patients. Fundings: RIN2018

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P08.051.A Microdeletions at 19p13.11 in four individuals with neurodevelopmental delay

Melissa Rieger[1], *Sébastien Moutton*[2], *Sarah Verheyen*[3], *Michael Speicher*[3], *Bruno Leheup*[4], *Celine Bonnet*[5], *Katharina Steindl*[6],

Beatrice Boneda[6], Anita Rauch[6], Mandy Krumbiegel[1], André Reis[1], Ulrike Hüffmeier[1]

[1]Human Genetics, University of Erlangen, Erlangen, Germany, [2]CPDPN, Pôle mère enfant, MSPBordeaux Bagatelle, Talence, France, [3]Institute of Human Genetics, Diagnostic and Research Center for MolecularBioMedicine, Medical University of Graz, Graz, Austria, [4]Service de génétique médicale, CHU de Nancy, Nancy, France, [5]Laboratoire de génétique médicale, CHRU Nancy, Nancy, France, [6]Institute of Medical Genetics, University of Zurich, Schlieren, Switzerland.

Introduction: Chromosomal microarrays (CMA) have been used to investigate the etiology of developmental disorders/intellectual disability (DD/ID) for more than a decade. The pangenomic approach allowed to identify numerous microdeletion and microduplication syndromes in undiagnosed patients and contributed to increased diagnostic rate and to better define genotype phenotype correlations. Few cases with copy number variants at 19p13.11 and scarce clinical information have been previously described. Most of them were identified by molecular genetic testing due to intellectual disability.

Material and methods: Using CMA, microdeletions at chromosome band 19p13.11 were detected in four independent individuals presenting with a DD/ID phenotype. The group was gathered through Decipher and internal collaborations.

Results: All four patients presented with variable neurodevelopmental and speech delay as well as behavioral and sleeping difficulties. Moreover, they partly showed cardiovascular, skeletal, and various other malformations. Most common overlapping dysmorphic features were a prominent nose, a thin upper vermilion, and a short neck. Dysmorphic features were rather subtle and non-specific and therefore not considered as a recognizable facial gestalt. The analyzed parents did not carry the deletions, indicating a de novo occurrence. The deletion sizes ranged between 0.7 – 1.5 Mb, located between Megabases 18-21 and contained a variable number of protein-coding genes (n = 25-48). For none of the genes in the shortest region of overlap there was enough evidence to qualify them as candidates for the neurodevelopmental delay.

Conclusion: Our findings support a locus on 19p13.11 associated with neurodevelopmental disorder and variable malformations.

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P08.052.B *EIF3F*-related neurodevelopmental disorder: refining the phenotypic and expanding the molecular spectrum

*Ulrike D. Hüffmeier[1], Cornelia Kraus[1], Miriam S. Reuter[1], Steffen Uebe[1], Mary-Alice Abbott[2], Syed A. Ahmed[3], Kristyn L. Rawson[4], Eileen Barr[5], Hong Li[5], Ange-Line Bruel[6], Laurence Faivre[6], Frédéric Tran Mau Them[6], Christina Botti[7], Susan Brooks[7], Kaitlyn Burns[8], Isum Ward[8], Marina Dutra-Clarke[9], Julian A. Martinez-Agosto[9], Hane Lee[9], Stanley F. Nelson[9], UCLA California Center for Rare Disease[9], Pia Zacher[10], Rami Abou Jamra[10], Chiara Klöckner[10], Julie McGaughran[11], Jürgen Kohlhase[12], Sarah Schuhmann[1], Ellen Moran[13], John Pappas[13], Annick Raas-Rothschild[14], Maria J. Guillen Sacoto[15], Lindsay B. Henderson[15], Timothy Blake Palculict[15], Sureni V. Mullegama[15], Houda Zghal Elloumi[15], Adi Reich[15], Samantha A. Schrier Vergano[16], Erica Wahl[17], André Reis[1], **Christiane Zweier**[1,18]*

[1]Human Genetics, University of Erlangen, Erlangen, Germany, [2]Medical Genetics, Department of Pediatrics, University of Massachusetts Medical School – Baystate, Springfield, MN, USA, [3]Department of Genetics, Southern California Permanente Medical Group, Kaiser Permanente, Riverside, CA, USA, [4]Department of Genetics, Southern California Permanente Medical Group, Kaiser Permanente, Riverside, MN, USA, [5]Department of Human Genetics, Emory University School of Medicine, Atlanta, GA, USA, [6]UMR-Inserm 1231 GAD team, Génétique des Anomalies du développement, Université de Bourgogne Franche-Comté, Dijon, France, [7]Division of Medical Genetics, Department of Pediatrics, Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ, USA, [8]Sanford Health, Sioux Falls, SD, USA, [9]Division of Genetics, Department of Pediatrics, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, CA, USA, [10]Institute of Human Genetics, University of Leipzig Medical Center, Leipzig, Germany, [11]Genetic Health Queensland, Royal Brisbane and Woman's Hospital, Brisbane and School of Medicine, The University of Queensland, St Lucia, Brisbane, Australia, [12]Synlab Human Genetics Freiburg, Freiburg, Germany, [13]Clinical Genetics, Hassenfeld Children's Hospital at NYU Langone, NYU Langone, Orthopedic Hospital, New York, NY, USA, [14]Sackler School of Medicine at Tel Aviv University, Tel Aviv, Israel, and Institute of Rare Diseases, Edmond & Lily Safra Children Hospital, Tel Hashome, Italy, [15]GeneDx, Gaithersburg, MD, USA, [16]Division of Medical Genetics and Metabolism, Children's Hospital of The King's Daughters, Norfolk, VA, USA, [17]UBMD Pediatrics, Division of Genetics, Buffalo, NY, USA, [18]Institut of Human Genetics, Universitätsspital Bern, Bern, Switzerland.

Introduction: An identical homozygous missense variant in EIF3F, identified through a large-scale genome-wide sequencing approach, was reported as causative in nine individuals with a neurodevelopmental disorder, characterized by variable intellectual disability, epilepsy, behavioral problems and sensorineural hearing-loss.

Material and methods: To refine the phenotypic and expand the molecular spectrum of EIF3F-related neurodevelopmental disorder, we examined independent patients.

Results: 21 patients were homozygous and one compound heterozygous for c.694T>G/p.(Phe232Val) in EIF3F. Haplotype analyses in 15 families suggested that c.694T>G/p.(Phe232Val) was a founder variant. All affected individuals had developmental delays including delayed speech development. About half of the affected individuals had behavioral problems, altered muscular tone, hearing loss, and short stature. Moreover, this study suggests that microcephaly, reduced sensitivity to pain, cleft lip/palate, gastrointestinal symptoms and ophthalmological symptoms are part of the phenotypic spectrum. Minor dysmorphic features were observed, although neither the individuals' facial nor general appearance were obviously distinctive. Symptoms in the compound heterozygous individual with an additional truncating variant were at the severe end of the spectrum in regard to motor milestones, speech delay, organic problems and pre- and postnatal growth of body and head, suggesting some genotype-phenotype correlation.

Conclusions: Our study refines the phenotypic and expands the molecular spectrum of EIF3F-related syndromic neurodevelopmental disorder. Funding: IZKF project E31 to CZ, NIH National Center for Advancing Translational Science (NCATS) UCLA Clinical and Translational Science Institute (CTSI) grant number UL1TR001881.

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P08.053.C Investigating consanguineous families from Turkey to identify autosomal recessive neurodevelopmental disorders

Esen Gumuslu[1], *Evren Gümüs*[2], *Ozlem Oz*[3], *Melis Ozkan*[4], *Kadri Karaer*[5], *Arif Ekici*[1], *Edibe Pembegul Yildiz*[4], *Nur Aydinli*[4], *André Reis*[1]

[1]*Institute of Human Genetics University Hospital Friedrich-Alexander-University of Erlangen-Nürnberg, Erlangen, Germany,* [2]*Sitki Kocman University School of Medicine, Medical Genetics Department, Mugla, Turkey,* [3]*Harran University School of Medicine, Medical Genetics Department, Sanliurfa, Turkey,* [4]*Istanbul University School of Medicine, Pediatric Neurology Department, Istanbul, Turkey,* [5]*Pamukkale University School of Medicine, Medical Genetics Department, Denizli, Turkey.*

Introduction: Neurodevelopmental disorders(NDDs) are genetically and phenotypically highly heterogeneous. Autosomal recessive (AR) genetic defects are more difficult to diagnose and are thus underreported. In consanguineous marriages, AR gene alterations are enriched, but these families also can have autosomal dominant new mutations and X-linked inherited variants. We reasoned that studying families with multiple affected individuals would select for AR inheritance.

Materials and Methods: We recruited 176 patients from 77 consanguineous families with NDDs with two or more affected individuals through clinical genetic and neuro-paediatric consultations from various academic hospitals in Turkey. In a pilot study, we report exome sequencing results from a subset of 17 families with 38 affected.

Results: In 12 individuals (32%) from six families we identified previously described pathogenic or likely pathogenic variants in six genes (ADAT3, CLP1, KMT2D, NSUN, PTRH2 and SCNA8A). In 24 individuals we found rare missense variants in established or candidate genes. Of these, in 9 individuals the phenotype was in accordance with the literature, bringing number of "solved" cases to 21 (55%). In 14 individuals we found multiple rare variants in genes causing overlapping phenotypes, which makes these cases difficult to disambiguate. In three families, affected siblings were discordant for the molecular findings.

Conclusion: This study has identified novel variants in known (candidate) genes in 55% of individuals allowing broadening of the phenotypic spectrum associated with these genes. The majority of variants are missense and difficult to interpret. Affected individuals often have variants in multiple genes, raising the question of possibly blended phenotypes.

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P08.054.D High diagnostic rate in publicly funded clinical whole exome sequencing for neurodevelopmental disorders and congenital anomalies: A tertiary center experience with 280 probands

Ben Pode-Shakked[1,2], *Oratl Barel*[1], *Amihood Singer*[3], *Elon Pras*[1,2], ***Lior Greenbaum***[1,2]

[1]*Sheba Medical Center, Tel Hashomer, Israel,* [2]*Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel,* [3]*Community Genetics, Public Health Services, Ministry of Health, Jerusalem, Israel.*

Whole exome sequencing (WES) is an important diagnostic tool for individuals affected by neurodevelopmental disorders (NDD) and/or multiple congenital anomalies (MCA). However, WES cost is often an obstacle for pursuing this option. We evaluated the yield of publicly funded clinical WES, performed in a single tertiary referral center during a three-year period (2018-2020). All index cases had the following: (1) moderate to severe intellectual disability (ID) (intelligence quotient [IQ]/developmental quotient [DQ] ≤55); or (2) mild to moderate ID (IQ/DQ<70) with epilepsy or congenital anomaly; or (3) MCA. Only subjects with normal chromosomal microarray analysis results who met inclusion criteria, were offered to participate. Overall, 280 consecutive families were included. In 250 (89.3%) families, the index case had NDD. In 252 of the families (90.0%), a trio WES was performed. Molecular diagnosis was reached in 115 (41.1%) families, mainly due to de novo mutations (92/115, 80.0%). Disease-causing variants were identified in a total of 102 genes, fifteen of which were implicated in more than a single family. Both paternal and maternal age at pregnancy were older in families with a de novo mutation, compared to all other cases. Yield was lower in families with premature birth compared to birth at term. Other demographic and clinical variables (including multiply affected family, coexistence of epilepsy, autism spectrum disorder, abnormal brain imaging or microcephaly) were not significantly associated with WES yield. Taken together, our findings support WES utility in a real-world setting, as part of a publicly funded genetic work-up for NDD and/or MCA.

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P08.055.A *NFIB* - associated intellectual disability and/or speech delay: first report of two novel structural variant disruptions

Constantia Aristidou[1,2], *Marselia Pantelidou*[1,2], ***LUDMILA KOUSOULIDOU***[1,2], *Athina Theodosiou*[1,2], *Ioannis Papaevripidou*[1], *Sofia Kitsiou - Tzeli*[3], *Niels Tommerup*[4], *Carolina Sismani*[1]

[1]*Department of Cytogenetics and Genomics, The Cyprus Institute of Neurology and Genetics, NICOSIA, Cyprus,* [2]*The Cyprus School of Molecular Medicine, Nicosia, Cyprus,* [3]*Department of Medical Genetics, Medical School, University of Athens, Athens, Greece,* [4]*Wilhelm Johannsen Centre for Functional Genome Research, Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark.*

The nuclear factor I (NFI) genes encode a family of transcription factors essential for multi-organ development during embryogenesis. *NFIA* and *NFIX* have been implicated in abnormal clinical phenotype manifestation. *NFIB* haploinsufficiency, caused by microdeletions and point mutations, has only recently been reported in patients with variable intellectual disability, macrocephaly, motor and speech delay. However, *NFIB* disruptions caused by structural variants have not been reported. Here we report two independent cases with *NFIB* disruptions that were identified through low-coverage whole-genome mate-pair sequencing (WG-MPS).

Specifically, WG-MPS was applied to map breakpoints in a female with dysmorphic facial features, speech delay and a

balanced t(4;9)(q26;p24)dn (patient 1), and a male with intellectual disability and an inv(9)(p22q21.2) inherited from his father with developmental and speech delay (patients 2,3). PCR primers flanking the predicted rearrangement junctions and Sanger sequencing were used to potentially identify clinically-relevant genes at the breakpoint sites.

The rearrangement breakpoints were refined to the base-pair level in all affected individuals. The derivative 9 breakpoint (chr9:14104379-14104385) in patient 1 directly disrupted *NFIB* intron 10, while the inv(9) breakpoint (chr9:14451707-14451711), identically found in patients 2,3, mapped ~138kb upstream *NFIB* (NM_001190737.2) (hg38). The remaining breakpoints were located in intergenic regions.

In conclusion, this study reports for the first time two cases with overlapping phenotypes carrying structural variants that disrupt directly or indirectly the *NFIB* gene. Future functional studies will define the underlying molecular mechanisms and further support the impact of these novel findings, thus expanding the current literature on the heterogeneous pathogenicity of *NFIB* variants.

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P08.056.B A case report of O-Donnell-Luria-Rodan syndrome with a novel truncating variant

Pilar Carrasco Salas[1], Rosario Mateos Checa[2], Ana Serrano Mira[1], ***Marina Oliva de Agar[3]***

[1]Genetic Unit, Clinical Analysis Department, Hospital Juan Ramón Jiménez, Huelva, Spain, [2]Neuropediatric Unit, Pediatric Department, Hospital Juan Ramón Jiménez, Huelva, Spain, [3]Genetics, Reference Laboratory, Barcelona, Spain.

Introduction: O'Donnell-Luria-Rodan (OLR) syndrome is a neurodevelopmental disorder described recently. In the only study published to date, a possible correlation between genotype and phenotype has been proposed. Most individuals with protein-truncating variants in *KMT2E* gene appear to present with generally mild developmental delay and intellectual disability, while patients with missense variants presented with the most severe phenotype.

Material and methods: The proband is a girl aged 6 years. In the first months of life, she presented with hypotonia, macrocephaly and three venous hemangiomas that made suspect initially a neurocutaneous syndrome. At age one, she started with treatment refractory epilepsy. Later, gastrointestinal abnormalities, progressive cerebral atrophy and severe development delay (she was unable to walk or speak) was observed. Due to this complex phenotype, whole exome sequencing was performed by SureSelectXT Human All Exon V5 (Agilent Technologies, USA). Parents' analysis was done by Sanger sequencing.

Results: A heterozygous frameshift variant was identified in *KMT2E* gene in the proband ((NM_182931.2): c.3487_3488insAT (p.Arg1163Hisfs*31)). It is a novel variant not previously described. Parents were not carried, being the diagnosis of OLR syndrome confirmed.

Conclusions: We present a case of severe OLR syndrome in a patient with a truncating variant. This suggests that the divergence in phenotype of patients with this syndrome is not related to the type of mutations identified, but is more likely a result of additional genetic or epigenetic factors. The patient had hemangiomas, a clinical feature not described in other patients until now. Further studies are required to confirm these two findings

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P08.057.C Recessively inherited pathogenic P4HTM gene variants cause Hypotonia, Hypoventilation, Intellectual Disability, Dysautonomia, Epilepsy, and Eye Abnormalities (HIDEA syndrome)

Elisa Rahikkala[1,2,3]*, Matti Myllykoski[4,5], Reetta Hinttala[2,4], Päivi Vieira[2,6], Naemeh Nayebzadeh[2,4], Simone Weiss[7], Astrid S. Plomp[8], Reginald Bittner[9], Mitja I. Kurki[10,11,12], Outi Kuismin[1,2,10], Andrea M. Lewis[13,14], Marja-Leena Väisänen[15], Hannaleena Kokkonen[15], Jonne Westermann[8], Gűnther Bernert[7], Hannu Tuominen[16], Aarno Palotie[10,11,12], Lauri Aaltonen[17], Yaping Yang[14,18], Lorraine Potocki[13,14], Jukka Moilanen[1,2], Silvana van Koningsbruggen[8], Xia Wang[14,18], Wolfgang M. Schmidt[9], Peppi Koivunen* contributed equally[4,5], Johanna Uusimaa* contributed equally[2,4,6]*

[1]Department of Clinical Genetics, Oulu University Hospital, Oulu, Finland, [2]PEDEGO Research Unit and Medical Research Centre Oulu, University of Oulu and Oulu University Hospital, Oulu, Finland, [3]Institute of Biomedicine, University of Turku, Turku, Finland, [4]Biocenter Oulu, University of Oulu, Oulu, Finland, [5]Faculty of Biochemistry and Molecular Medicine, Oulu Centre for Cell-Matrix Research, University of Oulu, Oulu, Finland, [6]Department of Children and Adolescents, Division of Paediatric Neurology, Oulu University Hospital, Oulu, Finland, [7]Kaiser Franz Josef Hospital with G.v. Preyer Children's Hospital, Department of Pediatrics, Vienna, Austria, [8]Department of Clinical Genetics, Amsterdam UMC, University of Amsterdam, Amsterdam, Netherlands, [9]Neuromuscular Research Department, Medical University of Vienna, Centre for Anatomy and Cell Biology, Vienna, Austria, [10]Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Helsinki, Finland, Helsinki, Finland, [11]Psychiatric & Neurodevelopmental Genetics Unit, Massachusetts General Hospital, Boston, MA, USA, [12]The Stanley Center for Psychiatric Research, The Broad Institute of MIT and Harvard, Cambridge, MA, USA, [13]Texas Children's Hospital, Houston, TX, USA, [14]Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA, [15]Northern Finland Laboratory Centre NordLab and Medical Research Centre, Oulu University Hospital and University of Oulu, Oulu, Finland, [16]Department of Pathology, Oulu University Hospital, Oulu, Finland, [17]Department of Medical Genetics, Genome-Scale Biology Research Program, University of Helsinki and Haartman Institute, Helsinki, Finland, [18]Baylor Genetics, Houston, TX, USA.

Introduction: A transmembrane prolyl 4-hydroxylase (P4H-TM) encoded by the *P4HTM* gene has not yet been associated with any Mendelian disorder. A new syndrome with hypotonia, intellectual disability and eye abnormalities (HIDEA) was recently described in a large consanguineous family from Northern Finland. Genome sequencing resulted in a shortlist of three candidate genes with potentially pathogenic biallelic variants: *TKT*, *P4HTM* and *USP4*. However, the causative gene remained elusive.

Material & methods: International collaboration and whole exome sequencing (WES) analysis were used to identify new patients with HIDEA and biallelic potentially pathogenic variants in the *P4HTM* gene. P4H-TM wild type and variant constructs without the transmembrane region were overexpressed in insect cells and analyzed with SDS-PAGE and Western blot.

Results: Five different homozygous or compound heterozygous pathogenic *P4HTM* gene variants were identified in six new and six previously published patients presenting with HIDEA. Hypoventilation, obstructive and central sleep apnea and dysautonomia were identified as novel features associated with the phenotype. Characterization of three of the P4H-TM variants demonstrated insoluble protein products and, thus, loss-of-function.

Conclusions: Biallelic loss-of-function *P4HTM* variants were shown to cause HIDEA syndrome. Our findings enable diagnosis of the condition, and highlight the importance of assessing the need for non-invasive ventilatory support in patients. Funding: This study was supported by the Academy of Finland Grants 266719 and 308009, the S. Jusélius Foundation, the Emil Aaltonen Foundation and the Jane and Aatos Erkko Foundation to P.K.

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P08.058.D Phelan-McDermid syndrome: the use of modern methods of cytogenetic examination in the diagnosis of autism spectrum disorders

Larysa Sheiko[1], *Iryna Lastivka*[2], *Vita Antsupova*[3], *Iana Ushko*[3], *Ljudmila Brisevac*[1], *Volodymyr Davydiuk*[4]

[1]*Shupyk National Medical Academy of Postgraduate Education, Kyiv, Ukraine,* [2]*Bukovinian State Medical University, Chernivtsi, Ukraine,* [3]*Bohomolets National Medical University, Kyiv, Ukraine,* [4]*National Pirogov Memorial Medical University, Vinnitsa, Ukraine.*

Introduction: Phelan-McDermid syndrome (PMD) is one of the autism spectrum disorder (ASD) syndromes caused by the deletion of the terminal or interstitial parts chromosome 22q13.3. In the case of the formation of a circular chromosome without loss of material, the phenotype remains normal, but there is a risk of microdeletion in the offspring. Patients with PMD are usually seen with a diagnosis of undifferentiated mental retardation or autism.

Materials and Methods: a clinical case of Phelan-McDermid syndrome in a child with undifferentiated mental retardation. Clinical genealogical, syndromic, cytogenetic, molecular genetic methods were used.

Results: A six-year-old girl with undifferentiated mental retardation was referred for genetic counseling. Previously observed by a pediatrician, pediatric neurologist, psychiatrist for microcephaly, delayed statokinetic and psychoverbal development. Girl phenotype: dolichocephaly, high forehead, flattening of the middle part of the face, deep-set eyes, full and puffy eyelids, long eyelashes, hypertelorism, full cheeks, enlarged ears. The child exhibits autistic behavior. Genetic testing included determination of the karyotype of the proband and parents by several methods: GTG; FISH with DNA samples WCP1-22, X, Y and FISH with locus specific samples 22SI LSI TUPLE1, 22q13 ARSA. Result: 46,XX,r(22)(p11.2q13), Phelan McDermid syndrome, recommendations for the rehabilitation of the child were given. Maternal karyotype: 46, XX. Paternal karyotype: 46,XY,r(22), gene sequencing is recommended SHANK3.

Conclusions: The use of a complex of modern cytogenetic methods, FISH with DNA probes, or SHANK3 gene sequencing can significantly increase the number of diagnosed cases of genetically determined mental retardation and increase the effectiveness of preventive measures.

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P08.063.A Expanding the phenotype of QRICH1 associated neurodevelopmental disorder

Smitha Kumble[1], *Amanda M. Levy*[2], *Sixto García-Miñaúr*[3,4], *Hua Gao*[5], *Maria Palomares-Bralo*[3,4], *Marta Pacio-Míguez*[3], *Sumit Parikh*[6], *Alyssa L. Ritter*[7], *Kosuke Izumi*[7], *Trevor L. Hoffman*[8], *Henry Oppermann*[9], *Lise Bjerglund*[10], *Jill A. Rosenfeld*[11], *Cynthia Curry*[12], *Laurence Faivre*[13,14], *Anja Leiber*[15], *Scott Robinson*[16], *Richard S. Finkel*[17,18], *Amanda Gerard*[11,19], *Julie S. Cohen*[20], *Yili Xie*[21], *Sureni V. Mullegama*[21], *Konrad Platzer*[9], *Maria J. Guillen Sacoto*[21], *Anya Revah-Politi*[22,23], *Sara M. Berger*[24], *Anne M. Comi*[25], *Kwame Anyane-Yeboa*[16], *Alice Fiévet*[26,27], *Cassie S. Mintz*[28], *Reymundo Lozano*[29], *Jennifer E. Posey*[11], *Michael Ciliberto*[30], *Lillian Howard*[30], *Paul Benke*[31], *Sylvie Odent*[32], *Anne-Sophie Denommé-Pichon*[33,34], *Mathys Wéber*[13], *Nicholas Ah Mew*[35], *Elsebet Ostergaard*[36,37], *Alejandro D. Iglesias*[38], *Jaya Punetha*[11,39], *Wendy K. Chung*[40], *Natasha Brown*[1,41], *Zeynep Tümer*[2,37]

[1]*Victorian Clinical Genetics Services, Murdoch Children's Research Institute, Melbourne, Australia,* [2]*Kennedy Center, Department of Clinical Genetics, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark,* [3]*Institute of Medical and Molecular Genetics, La Paz University Hospital, Idipaz, Madrid, Spain,* [4]*Centro de Investigación Biomédica en Red de Enfermedades Raras, Instituto Carlos III, Madrid, Spain,* [5]*Department of Review Analysis, GeneDx LLC, Maryland, MD, USA,* [6]*Mitochondrial Medicine & Neurogenetics, Cleveland Clinic, Cleveland, OH, USA,* [7]*Divison of Human Genetics, Department of Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA, USA,* [8]*Regional Department of Genetics, Southern California Kaiser Permanente Medical Group, Pasadena, CA, USA,* [9]*Institute of Human Genetics, University of Leipzig Medical Center, Leipzig, Germany,* [10]*Department of Pediatrics, University Hospital Hvidovre, Copenhagen, Denmark,* [11]*Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX, USA,* [12]*Genetic Medicine, Dept of Pediatrics, UCSF/Fresno, Fresno, CA, USA,* [13]*Centre de Référence Anomalies du Développement et Syndromes Malformatifs, FHU TRANSLAD, Hôpital d'Enfants, CHU Dijon, Dijon, France,* [14]*Inserm UMR1231 GAD, Génétique des Anomalies du Développement, Université de Bourgogne, Dijon, France,* [15]*Department of neuropediatrics, Childrens Hospital of Eastern Switzerland, St. Gallen, Switzerland,* [16]*Department of Pediatrics, Columbia University New York, New York, NY, USA,* [17]*Nemours Children's Hospital, Orlando, FL, USA,* [18]*Center for Experimental Neurotherapeutics, St. Jude Children's Research Hospital, Memphis, TN, USA,* [19]*Texas Children's Hospital, Houston, TX, USA,* [20]*Department of Neurology and Developmental Medicine, Kennedy Krieger Institute, Baltimore, MD, USA,* [21]*Clinical Genomics Program, GeneDx, Maryland, MD, USA,* [22]*Institute for Genomic Medicine, Columbia University Medical Center, New York, NY, USA,* [23]*Precision Genomics Laboratory, Columbia University Irving Medical Center, New York, NY, USA,* [24]*Departments of Pediatrics, Columbia University New York, New York, NY, USA,* [25]*Kennedy Krieger Institute Hugo Moser Research Institute, Departments of Neurology and Pediatrics, Johns Hopkins School of Medicine, Baltimore, MD, USA,* [26]*GCS SeqOIA, Paris, France,* [27]*Service Génétique des Tumeurs, Gustave Roussy, Villejuif, France,* [28]*Department of Genetics and Genomic Sciences and Pediatrics, Icahn School of Medicine at Mount Sinai Hospital, New York, NY, USA,* [29]*Department of Genetics and Genomic Sciences, Pediatrics and Psychiatry, Icahn School of Medicine at Mount Sinai Hospital, New York, NY, USA,* [30]*Stead Family Department of Pediatrics, University of Iowa, Iowa City, IA, USA,* [31]*Joe DiMaggio Children's Hospital, Hollywood, FL, USA,* [32]*CHU Rennes, Hôpital Sud, Service de Génétique Clinique, Univ Rennes, Centre de référence Anomalies du développement CLAD-Ouest, ERN ITHACA, Rennes, France,* [33]*INSERM UMR 1231*

Equipe GAD, Université de Bourgogne, Dijon, France, [34]Unité Fonctionnelle Innovation en Diagnostic génomique des maladies rares, FHU-TRANSLAD, CHU Dijon Bourgogne, Dijon, France, [35]Rare Disease Institute, Children's National Hospital, Washington, DC, USA, [36]Department of Clinical Genetics, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark, [37]Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, [38]Division of Clinical Genetics, Department of Pediatrics, Columbia University Irving Medical Center, New York, NY, USA, [39]Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA, [40]Departments of Pediatrics and Medicine, Columbia University New York, New York, NY, USA, [41]University of Melbourne Department of Paediatrics, Royal Children's Hospital, Melbourne, Australia.

Introduction: Nine *de novo* loss-of-function and a single missense variant in the *QRICH1* gene have previously been reported in individuals with developmental delay, autism, short stature, facial dysmorphism and chondrodysplasia. We present 27 additional individuals with *QRICH1*-related neurodevelopmental disorder to further delineate the spectrum of clinical features and genetic variants contributing to this emerging autosomal dominant syndrome.

Methods: Phenotypic and molecular data from 27 previously unreported individuals with *QRICH1* variants were gathered through international collaboration and compared to those of the 10 previously reported individuals.

Results: Frequent phenotypic features included mild to moderate developmental delay/intellectual disability (70%), facial dysmorphism (84%), short stature (41%), poor weight gain (41%) and hypotonia (49%). Additional findings were seizures (24%), minor structural brain abnormalities (24%) and scoliosis (19%). Twenty-seven individuals had truncating or splice variants, and 10 had missense variants. Four variants were inherited from a mildly affected parent. Individuals with missense variants were more likely to report early language delay as compared to those with loss-of-function variants (10/10 vs 14/27, $p\ value = 0.005$).

Conclusion: In addition to the known neurodevelopmental features and short stature, we expand the phenotypic spectrum of *QRICH1*-associated disorders to include poor weight gain, hypotonia, minor structural brain anomalies, scoliosis and seizures. Inherited variants from mildly affected parents are reported for the first time, suggesting variable expressivity. Aside from language delay, there were no other statistically significant phenotypic differences between individuals with missense variants as compared to loss-of-function variants. However, additional data are required to determine genotype/phenotype correlations.

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P08.064.B An asymptomatic male carrying a constitutional pathogenic MEPC2 variant, identified through his daughter with Rett syndrome

*Michelle Caudle[1], Jacqueline Ogilvie[2], John Vincent[3], Hanxin Lin[4], Rebecca Lau[3], Anna Mikhalov[3], **Victoria M. Siu**[1,5]*

[1]Medical Genetics Program of Southwestern Ontario, London Health Sciences Centre, London, ON, Canada, [2]Division of Developmental Pediatrics, Department of Pediatrics, Schulich School of Medicine and Dentistry, Western University, London, ON, Canada, [3]Family Mental Health Research Institute, CAMH, Toronto, ON, Canada, [4]Department of Pathology and Laboratory Medicine, Schulich School of Medicine and Dentistry, Western University, London, ON, Canada, [5]Division of Medical Genetics, Department of Pediatrics, Schulich School of Medicine and Dentistry, Western University, London, ON, Canada.

Introduction: Rett syndrome, an X-linked neurodevelopmental disorder typically affecting females, is most often caused by *de novo* mutations in *MECP2* on the paternally inherited allele. Pathogenic hemizygous *MECP2* variants in males are usually embryonic lethal or cause severe neonatal encephalopathy. Case: A female child, born at 29 weeks with uncomplicated neonatal course, presented at 1 year with regression in babbling and lower limb spasticity. By 36 months she remained non-verbal and displayed mid-line stereotypies. Genetic testing identified a heterozygous pathogenic variant in the *MECP2* gene, NM_001110792.1: c.1195_1246del; p.(Pro399Serfs*5). This 52 bp deletion in the last exon is expected to result in a frameshift with premature termination of protein synthesis. Unexpectedly, her unaffected father was found to be hemizygous for this variant in DNA from blood, buccal swab, saliva, and urine. His unaffected mother and sister were heterozygous for the variant. Trio whole exome sequencing of the proband and her parents confirmed the *MECP2* variant but did not identify any additional pathogenic variants in other genes. Inexplicably, mRNA analysis of the proband's DNA only yielded PCR products amplified from the mutant allele while in the proband's father, there was no *MECP2* mRNA amplification at all.

Conclusions: While we believe that the frameshift variant is pathogenic in the proband, we cannot explain why her father is unaffected. We postulate that the C terminal deletion may have variable effects or that there may be some modifier gene(s) involved. This is the first report of an unaffected male carrying a constitutional mutation in *MECP2*.

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P08.065.C *SEMA6B* variants cause Intellectual Disability and alter dendritic spine density in mouse primary hippocampal neurons

Amélie Cordovado[1], *Médéric Jeanne[1,2], Veranika Panasenkava[1], Anne-Sophie Denommé-Pichon[3], Boris Keren[4], Cyril Mignot[4], Lance Rodan[5], Keri Ramsey[6], Vahid Bahrambeigi[7], Wendy Mitchell[8], Jillian R Ozmore[9], Leslie Granger[10], Andrea Petersen[10], Paige Matheny[11], Margaret Au[11], Chanika Phornphutkul[12], Joaquín Alejandro Fernández-Ramos[13], Eduardo Lopez-Laso[13], Marcella Zollino[14], Manuela Morleo[15], Telethon Undiagnosed Diseases Program, Rebecca Herzog[16], Mona Grimmel[17], David Mei[18], Renzo Guerrini[18], Richard Redon[19], Stéphane Bézieau[20], Consortium HUGODIMS, Frédéric Laumonnier[1], Annick Toutain[1,2], Marie-Laure Vuillaume[1,2]*

[1]UMR 1253, iBrain, University of Tours, Inserm, Tours, France, [2]Genetics Department, University Hospital of Tours, Tours, France, [3]Genetics Department, Hospital of Dijon, Dijon, France, [4]Genetics Department, Hospital group Pitié-Salpêtrière, APHP, Paris, France, [5]Department of Pediatrics, Boston Children's Hospital, Boston, MA,

USA, [6]Center for Rare Childhood Disorders, Translational Genomics Research Institute, Phoenix, AZ, USA, [7]Graduate Program in Diagnostic Genetics, School of Health Professions, The University of Texas MD Anderson Cancer Center, Houston, TX, USA, [8]Neurology Division Children's Hospital Los Angeles, Los Angeles, CA, USA, [9]Department of Medical Genetics, Dartmouth-Hitchcock Medical Center, Lebanon, NH, USA, [10]Department of Rehabilitation and Development, Randall Children's Hospital at Legacy Emanuel Medical Center, Portland, OR, USA, [11]Division of Genetics and Metabolism, University of Kentucky HealthCare, Lexington, KY, USA, [12]Division of Human Genetics, Warren Alpert Medical School of Brown University, Hasbro Children's Hospital/Rhode Island Hospital, providence, RI, USA, [13]Pediatric Neurology Unit, Department of Pediatrics, University Hospital Reina Sofía, IMIBIC and CIBERER, Cordoba, Spain, [14]Institute of Genomic Medicine, Catholic University, Gemelli Hospital Foundation, Rome, Italy, [15]Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Naples, Italy, [16]Institute of Systems Motor Science, University of Lübeck, Center for Brain, Behavior and Metabolism, Ratzeburger Allee 160, 23538, Lübeck, Germany, [17]Institute of Medical Genetics and Applied Genomics, Hertie Institute for Clinical Brain Research, Tübingen, Germany, [18]Pediatric Neurology, Neurogenetics, and Neurobiology Unit and Laboratories, Meyer Children's Hospital-University of Florence, Florence, Italy, [19]INSERM, CNRS, Nantes University, University Hospital Center, Thorax Institute, Nantes, France, [20]Genetics Department, Hospital of Nantes, Nantes, France.

Introduction: Intellectual Disability (ID) is a common neurodevelopmental disorder frequently caused by monogenic defects. By conducting whole exome sequencing in a patient presenting with severe ID and after a call for international collaboration, we collected 11 *SEMA6B de novo* heterozygous variants carried by 14 unrelated patients. Clinical features in these patients include moderate to severe ID, poor language, movement disorder with ataxia, and sometimes seizures. To assess the involvement of SEMA6B in this phenotype, we initiated in vitro functional studies in HEK cells and in mouse primary hippocampal neurons.

Materials and Methods: Plasmids containing either the wild-type or three mutated forms of *Sema6b* (V183L, R372* and W689*) were overexpressed first, in HEK293T cell line to assess the impact of *the* variants overexpression on Sema6b expression, stability and subcellular localization and then, in primary neuronal cultures to characterize the effect of *the* variants on morphogenesis and synaptogenesis.

Results: Sema6b stability and subcellular localization are altered only by overexpression of the R372* variant. This variant by leading to the formation of a truncated protein, decreases Sema6b expression and stability and prevents Sema6b from reaching the plasma membrane. Regarding neuronal cultures, the number of dendritic spines in particular mature spines is significantly decreased after overexpression of V183L and W689* variants.

Conclusion: Identification of *SEMA6B* variants in patients presenting with an overlapping phenotype combined with in vitro functional studies highlights the important role of SEMA6B in neuronal development notably in spine formation and maturation, and adds *SEMA6B* to the list of ID-related genes.

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P08.066.D Delineating the molecular and phenotypic spectrum of the SETD1B-related syndrome

*Marjolein J. A. Weerts[1], **Kristina Lanko**[1], Francisco J. Guzmán-Vega[2], Adam Jackson[3], Reshmi Ramakrishnan[2], Kelly J. Cardona-Londoño[2], Karla A. Peña-Guerra[2], Yolande van Bever[1], Barbara W. van Paassen[1], Anneke Kievit[1], Marjon van Slegtenhorst[1], Nicholas M. Allen[4], Caroline M. Kehoe[4], Hannah K. Robinson[5], Lewis Pang[5], Selina H. Banu[6], Mashaya Zaman[6], Stephanie Efthymiou[7], Henry Houlden[7], Irma Järvelä[8], Leena Lauronen[9], Tuomo Määttä[10], Isabelle Schrauwen[11], Suzanne M. Leal[11], Claudia A. L. Ruivenkamp[12], Daniela Q. C. M. Barge-Schaapveld[12], Cacha M. P. C. Peeters-Scholte[13], Hamid Galehdari[14], Neda Mazaheri[14], Genomics England Research Consortium, Sanjay M. Sisodiya[15], Victoria Harrison[16], Reza Maroofian[17], Siddharth Banka[3], Bekim Sadikovic[18], Stefan T. Arold[2], Tahsin Stefan Barakat[1]*

[1]Erasmus MC, Rotterdam, Netherlands, [2]King Abdullah University of Science and Technology (KAUST), Computational Bioscience Research Center (CBRC), Division of Biological and Environmental Sciences and Engineering (BESE), Thuwal, Saudi Arabia, [3]Manchester Centre for Genomic Medicine, St. Mary's Hospital, Manchester University NHS Foundation Trust, Health Innovation Manchester, Manchester, United Kingdom, [4]Department of Paediatrics, National University of Ireland Galway, Galway, Ireland, [5]Exeter Genomics Laboratory, RILD Building, Royal Devon and Exeter NHS Foundation Trust, Exeter, United Kingdom, [6]Department of Pediatric Neurology, Dr. M.R. Khan Shishu (Children) Hospital and ICH, Dhaka, Bangladesh, [7]Department of Neuromuscular Disorders, Queen Square Institute of Neurology, University College London, London, United Kingdom, [8]Department of Medical Genetics, University of Helsinki, Helsinki, Finland, [9]Department of Clinical Neurophysiology, New Children´s Hospital, HUS Diagnostic Center, University of Helsinki and Helsinki University Hospital (HUH), Helsinki, Finland, [10]Disability Services, Joint Authority for Kainuu, Kajaani, Finland, [11]Center for Statistical Genetics, Sergievsky Center, Taub Institute for Alzheimer's Disease and the Aging Brain, Department of Neurology, New York, NY, USA, [12]Department of Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands, [13]Department of Neurology, Leiden University Medical Center, Leiden, Netherlands, [14]Department of Genetics, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran, Islamic Republic of, [15]Department of Clinical and Experimental Epilepsy, UCL Queen Square Institute of Neurology, London, United Kingdom, [16]Wessex Clinical Genetics Service, Princess Anne Hospital, Southampton, United Kingdom, [17]Department of Neuromuscular Disorders, Queen Square Institute of Neurology, University College London, London, United Kingdom, [18]Molecular Genetics Laboratory, Molecular Diagnostics Division, London Health Sciences Centre, London, United Kingdom.

SETD1B encodes a lysine-specific histone methyltransferase that methylates histone H3 at position lysine-4 (H3K4me1, H3K4me2, H3K4me3) and thereby is involved in the regulation of gene expression. Pathogenic variants in *SETD1B* have been associated with a syndromic neurodevelopmental disorder including intellectual disability, language delay and seizures. To date, clinical features have been described for eleven patients with (likely) pathogenic *SETD1B* sequence variants. We perform an in-depth clinical characterization of a cohort of 36 unpublished individuals with *SETD1B* sequence variants, describing their molecular and phenotypic spectrum. By means of computational protein modelling we predict potential functional effect of *SETD1B* variants. Selected variants located in different functional domains of SETD1B were functionally tested using in vitro and genome-wide methylation assays, confirming *in silico* predictions. Our data present evidence for a loss-of-function mechanism of *SETD1B* variants, resulting in a core clinical phenotype of global

developmental delay, language delay including regression, intellectual disability, autism and other behavioral issues, and variable epilepsy phenotypes. Developmental delay appeared to precede seizure onset, suggesting SETD1B dysfunction impacts physiological neurodevelopment even in the absence of epileptic activity. Interestingly, males are significantly overrepresented, and thus we speculate that sex-linked traits could affect susceptibility to clinical penetrance and the clinical spectrum of *SETD1B* variants. Together, this work expands the phenotypic and molecular spectrum associated with *SETD1B* variants, provides insights into their functional effects and will ultimately facilitate the counseling regarding the clinical spectrum of newly diagnosed patients with the *SETD1B*-related syndrome.

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P08.067.A New cases from Spanish population with intragenic pathogenic variants in *SETD5* gene: refining the phenotype and expanding the genotype

María José Sánchez Soler[1], *Fernando Santos-Simarro*[2,3], *Sixto García-Miñaúr*[2,3], *Ana Teresa Serrano Antón*[1], *Verónica Seidel*[4], *Graciela Pi Castán*[5], *Marta Pacio-Míguez*[2], *María Palomares-Bralo*[2,3,6]

[1]*Medical Genetic Section, H. Clinic Universitary V. Arrixaca, IMIB-Arrixaca, El Palmar, Spain,* [2]*Instituto de Genética Médica y Molecular (INGEMM), Hospital Universitario La Paz, IdiPaz., Madrid, Spain,* [3]*CIBERER,Centro de Investigación Biomédica en Red de Enfermedades Raras, ISCIII, Madrid, Spain,* [4]*Medical Genetic Section, Gregorio Marañón Hospital, Madrid, Spain,* [5]*Consulta de Dismorfología, Hospital Universitario de la Ribera, Alzira, Valencia, Spain,* [6]*European Reference Network Congenital Malformations & Rare Mental Disability (ERN -ITHACA), Madrid, Spain.*

Introduction: Loss-of-function variants in *SETD5* gene cause the core phenotype of 3p25.3 microdeletion syndrome characterized by intellectual disability/autism, slow growth, dysmorphic features and malformations such as postaxial polydactyly, heart condition and genitourinary anomalies. However, the prognosis seems to be milder in cases with intragenic *SETD5* variants. Until now, only 15 cases have been described.

Material and methods: Descriptive retrospective collaborative study of Spanish children with *de novo* intragenic variants in *SETD5* gene.

Results: 9 cases collected (3 females/6 males). Mean age: 6 years old (1.5-15). Prenatal anomalies: 3/9 single umbilical artery, 2/9 growth retardation and nuchal edema. Postnatally: all showed some neurodevelopment disorder but two presented normal intelligence and the language skills improved gradually. 2/9 hypotonia, 1/9 seizures. Growth was normal in 8/9 cases; 3/9 microcephaly. Congenital malformations: 5/9 had heart disease, 2/9 genitourinary anomalies and 6/9 digital anomalies (2 polydactyly). The main facial features were: trianglar face, arched/thick/unusual eyebrows, low nasal bridge, anteverted/thick nares and long/smooth philtrum. Molecular data: variants were identified by exome sequencing; all of them were protein truncating variants: 4 nonsense, 4frameshift and one affecting a splice-donor site; 2/9 previously described.

Conclusions: Our results support that hypotonia and microcephaly are uncommon and neurological prognosis is much better in these cases than in the 3p25.3 deletion syndrome. Digital anomalies in addition to polydactyly were common so it must be taken into account.Thus, *SETD5* gene haploinsufficiency should be considered in the differential diagnosis of KBG, Cornelia de Lange and Coffin Siris syndrome.

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P08.069.C Investigation of two novel *SOX4* mutations found in patients with intellectual disability

Martin Grosse[1], *Tabib Dabir*[2], *Shane McKee*[2], *Stefanie Beck-Wödl*[3], *Eva Manka*[4], *Alma Küchler*[1], *Christel Depienne*[1], *Frank Kaiser*[1]

[1]*University Hospital Essen, Essen, Germany,* [2]*Northern Ireland Regional Genetics Service, Belfast, United Kingdom,* [3]*University Hospital Tübingen, Tübingen, Germany,* [4]*EZSE, Essen, Germany.*

Introduction: SOX4 (OMIM:184430) is a transcription factor with pleiotropic functions required for developmental processes such as corticogenesis. Like other SOX proteins, SOX4 contains a highly conserved high mobility group (HMG) domain that mediates DNA binding, bending and nuclear trafficking. Recently patients with pathogenic variants in *SOX4* have been reported for the first time. We aimed to investigate the effects on transcriptional activation of two novel pathogenic *de novo* variants in *SOX4* identified in patients with intellectual disability.

Material and Methods: Wildtype (wt) or mutant *SOX4* were co-expressed with the known cofactor *POU3F2* and transcriptional activation was tested using a luciferase reporter assay in HEK 293 cells. Proper expression of the *SOX4* constructs and *POU3F2* was verified by western blot.

Results: Contrary to wt SOX4, which was able to induce ectopic luciferase expression when co-expressed with POU3F2, this effect was abolished for both pathogenic SOX4 variants (Arg61Gln and Glu27*).

Conclusions: We report two novel pathogenic variants in *SOX4* abolishing the transcriptional activation of SOX4 in vitro. While the variant SOX4Glu27* leads to a premature stop and loss of the functional domains, SOX4Arg61Gln shows an exchange of an amino acid that is highly conserved in the HMG box of several proteins. Besides its localization within a predicted bipartite nuclear localization signal, previous investigations have provided functional evidence for the role of arginine 61 in DNA-binding affinity of SOX4. Whether the loss of transcriptional activity is caused by alterations of DNA binding or intracellular localization of SOX4 is currently investigated.

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P08.070.D Compound heterozygous SPATA5 variants in siblings with intellectual disability, hypotonia and autistic features

Daniela Avdjieva-Tzavella[1], *Slavena Atemin*[2], *Tihomir Todorov*[3], *Hadil Kathom*[1], *Trayan Delchev*[1], *Albena Todorova*[2]

[1]Department of Clinical Genetics, University Pediatrics Hospital, Medical University, Sofia, Bulgaria, Sofia, Bulgaria, [2]Genetic Medico-Diagnostic Laboratory "Genica", Department of Medical Chemistry and Biochemistry, Medical University, Sofia, Bulgaria, [3]Genetic Medico-Diagnostic Laboratory "Genica", Sofia, Bulgaria.

Introduction: Variants in spermatogenesis-associated protein 5 gene (SPATA5) are associated with "Epilepsy, Hearing Loss and Mental Retardation Syndrome". SPATA5 protein localizes predominantly in the mitochondria and is proposed to be involved in mitochondrial remodeling, ATP production and brain development.

Materials and Methods: A boy aged 11 years (Patient 1) was born the first child to non-consanguineous parents at term. He was referred to genetic evaluation because of the mental retardation, hypotonia and autistic features. Patient 2 was a 1-year-old girl and the younger sister of Patient 1.She was referred to genetic evaluation because of the developmental delay and low muscle tone. Metabolic and mitochondrial DNA testing results were normal in both patients. In an attempt to establish the diagnosis Whole exome sequencing was carried out.

Results: Both patients underwent Whole Exome Sequencing. The two siblings carried compound heterozygous mutations in the SPATA5 gene: c.554G>A(p.Gly185Glu) and c.1831C>T(p.Pro611-Ser). The variants were confirmed by Sanger sequencing.

Conclusions: Our results describe new, probably pathogenic variants in SPATA5 gene, and we confirm that bi-allelic pathogenic variants in SPATA5 cause a syndromic form of intellectual disability. Our study expands the clinical spectrum of SPATA5 mutations.

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P08.071.A *STAG1* gene heterozygous *de novo* variant in a patient with Angelman syndrome like phenotype

Kristi Rähn[1], *Kai Muru*[1,2], *Sander Pajusalu*[1,2], *Eve Õiglane-Šlik*[3], *Katrin Õunap*[1,2]

[1]Department of Clinical Genetics, United Laboratories, Tartu University Hospital, Tartu, Estonia, [2]Department of Clinical Genetics, Institute of Clinical Medicine, University of Tartu, Tartu, Estonia, [3]Children's Clinic, Tartu University Hospital, Tartu, Estonia.

STAG1 gene variants (OMIM: Mental retardation, autosomal dominant 47, #617635) belong to a group of cohesinopathies and are previously described only in few cases, as a cause of unspecific intellectual disability. *STAG1* mutations are shown to be milder than the phenotype associated with other cohesinopathies. In published cases, the constant features characterizing *STAG1* gene variants are developmental delay, recognizable facial gestalt and variable associated features.Case report: The proband was born at term by CS due to fetal hypoxia, her birth weight was 3220g, length 52cm, OFC 33cm (-1.75 SD). In late infancy, she had some feeding problems and excessive vomiting episodes. Speech delay was noticed at the second year and from the third, her speech and independent eating skills regressed. At the age of 4, she has developmental delay, important receptive and expressive speech impairment. Her phenotype resembles Angelman syndrome and she has marked microcephaly - OFC 44.5cm (-4 SD). She has peculiar stereotypical "dancing" movements with her hands (less with her feet). Result: Trio-based exome sequencing analysis revealed heterozygous high quality frameshift *de novo* insertion in *STAG1* gene NM_005862.2(STAG1):c.3288_3289insT p.(Thr1097Tyrfs*7). It is absent from ClinVar and gnomAD databases.

Conclusion: We report the patient with *STAG1* gene variant, with Angelman-like phenotype, stereotypical hand movements and regression of skills, which is not been described before. The number of described patients with *STAG1* variants is very small thus it's phenotypic spectrum may be wider than currently described. Funding: Estonian Research Council grant PRG471, MOBTP175

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P08.072.B Tatton-Brown-Brahman syndrome: a novel mutation in DNMT3A

Margarita Martínez-Atienza[1], *Susana García-Linares*[2], *Antonio Miguel Poyatos-Andújar*[2], *Irene Sofía Machado-Casas*[2], *Susana Pedrinaci-Rodríguez*[1], *María Luz Bellido-Díaz*[1], *Matías Pérez-Sánchez*[1]

[1]Hospital Universitario Virgen de las Nieves, Granada, Spain, [2]Hospital Universitario Clínico San Cecilio, Granada, Spain.

Introduction: Tatton-Brown-Rahman syndrome (TBRS) is a congenital overgrowth disorder associated with intellectual disability initially identified in 2014 and is caused by constitutive variants of the DNMT3A gene. The principal features are overgrowth and intellectual disability.

Material and Methods: We present a 13-years-old boy who present development delay with a age of 2 years for independent walking and of 2.5 years for first spoken words and attention deficit hyperactivity disorder. We performed a serial molecular test wich included normal karyotype and array-CGH analyses and negative results for fragile-X, finally the clinical exome sequencing (CES) analysis offered a de novo variant in DNMT3A.

Results: We found a not reported variant c.2114T>C, p.Ile705Thr in DNMT3A gene, this variant was determined as *de novo* with the segregation analysis and is classified as likely pathogenic according to the ACMG criteria (PM1, PM2, PP2, PP3).

Conclusions: The phenotype of TBRS included intellectual disability and overgrowth, with frequent clinical associations included joint hypermobility, obesity, hypotonia, behavioural/psychiatric issues, kyphoscoliosis and afebrile seizures. TBRS overlaps clinically with other overgrowth intellectual disability síndromes. The majority of individuals with TBRS are healthy, however the bibliography reports possible complications of behavioural/psychiatric issues, kyphoscoliosis, febrile seizures, cardiac anomalies, hypotonia and/or joint hypermobility, and possibility of haematological malignancy. In our case, we have developed a care protocol together with the different medical specialties.

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P08.073.C Heterozygous Loss of Function Variants in TBCK Cause a Mild Neurologic Syndrome in Humans and Mice

Abdias Diaz-Rosado

Children's Hospital of Philadelphia, Philadelphia, PA, USA.

TBCK-related encephalopathy syndrome is a rare pediatric neurodegenerative disorder with no current treatment. The disease is characterized by loss-of-function biallelic mutations in *TBCK*, which leads to a downregulation of mTORC1 signaling and severe changes in brain morphology. Although formal studies on heterozygous carriers have never been done in the past, families

have reported differences. Therefore, in this study we used our heterozygous mice model (tbck+/-) to test this hypothesis. Both of our behavioral and molecular data suggest that there is a persistent neurologic phenotype, which could explain the differences observed in humans. Moreover, our group has also shown that heterozygosity in mice can affect autophagic flux (LC3-I/II), mTORC1 signaling (pS6 and downstream effectors) and even pup vocalizations at PND-6. This suggests the possibility that haploinsufficiency of TBCK can have phenotypic effect in human hets. Also, preliminary data from a large dataset indicates the presence of a statistically significant preponderance of neurologic symptoms in heterozygous humans. Although more information is still needed to understand these mechanisms, our results suggest that in heterozygous animals TBCK functions are affected and this can have further effects in metabolism and behavior. Hopefully in the near future we will be able to understand the basis for these pathologies and to establish the connection that exists between heterozygosity in animals and the presence of a phenotype in humans.

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P08.075.A Beyond founder and truncting variants in *TECPR2*-associated disorder

Sonja Neuser[1], *Barbara Brechmann*[2,3], *Gali Heimer*[4,5], *Ines Brösse*[3], *Susanna Schubert*[1], *Lauren O'Grady*[6], *Michael Zech*[7,8], *Siddharth Srivastava*[2], *David A. Sweetser*[6], *Yasemin Dincer*[9,10], *Volker Mall*[9,11], *Juliane Winkelmann*[7,8,12,13], *Christian Behrends*[13], *Basil T. Darras*[14], *Robert J. Graham*[15], *Parul Jayakar*[16], *Barry Byrne*[17], *Bat El Bar-Aluma*[4,5], *Yael Haberman*[4,5,18], *Amir Szeinberg*[4,5], *Hesham M. Aldhalaan*[19], *Mais Hashem*[20], *Amal A. Tenaiji*[21], *Shahnaz Ibrahim*[22], *Fatima Khan*[22], *Henry Houlden*[23], *Vijayalakshmi S. Ramakumaran*[24], *Alistair T. Pagnamenta*[25], *Jennifer E. Posey*[26], *James R. Lupski*[26,27,28,29], *Wen-Hann Tan*[30], *Gehad ElGhazali*[21], *Isabella Herman*[26,28,31], *Tatiana Muñoz*[32], *Gabriela M. Repetto*[32], *Angelika Seitz*[33], *Mandy Krumbiegel*[34], *M. Cecilia Poli*[27,32], *Usha Kini*[24], *Stephanie Efthymiou*[23], *Jens Meiler*[35,36], *Reza Maroofian*[23], *Fowzan S. Alkuraya*[20,37], *Rami Abou Jamra*[1], *Bruria Ben Zeev*[4,5], *Darius Ebrahimi-Fakhari*[2], *Bernt Popp*[1]

[1]*Institute of Human Genetics, University of Leipzig Medical Center, Leipzig, Germany,* [2]*Department of Neurology, The F.M. Kirby Neurobiology Center, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA,* [3]*Department of Pediatrics, Hospital for Children and Adolescents, Heidelberg University Hospital, Heidelberg, Germany,* [4]*Edmond and Lily Safra Children's Hospital, Sheba Medical Center, Ramat Gan, Israel,* [5]*Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel,* [6]*Division of Medical Genetics and Metabolism, Department of Pediatrics, Massachusetts General Hospital, Boston, MA, USA,* [7]*Institute of Neurogenomics, Helmholtz Zentrum München, München, Germany,* [8]*Institute of Human Genetics, Klinikum rechts der Isar, Technical University of Munich, München, Germany,* [9]*Social Pediatrics, Department of Pediatrics, Technische Universität München, München, Germany,* [10]*Zentrum für Humangenetik und Laboratoriumsdiagnostik (MVZ), Martinsried, Germany,* [11]*kbo-Kinderzentrum München, München, Germany,* [12]*Lehrstuhl für Neurogenetik, Technische Universität München, München, Germany,* [13]*Munich Cluster for Systems Neurology (Synergy), Ludwig-Maximilians-Universität München, München, Germany,* [14]*Department of Neurology, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA,* [15]*Department of Anesthesia, Critical Care and Pain Medicine, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA,* [16]*Nicklaus Children's Hospital, Miami, FL, USA,* [17]*Powell Gene Therapy Center, University of Florida, Gainesville, FL, USA,* [18]*Cincinnati Children's Hospital Medical Center and the University of Cincinnati College of Medicine, Cincinnati, OH, USA,* [19]*Department of Neuroscience, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia,* [20]*Department of Translational Genomics, Center for Genomic Medicine, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia,* [21]*Sheikh Khalifa Medical City, Abu Dhabi, United Arab Emirates,* [22]*Department of Paediatrics and Child Health, Aga Khan University Hospital, Karachi, Pakistan,* [23]*Department of Neuromuscular Disorders, Queen Square Institute of Neurology, University College London, London, United Kingdom,* [24]*Oxford Centre for Genomic Medicine, Oxford, United Kingdom,* [25]*NIHR Biomedical Research Centre, Wellcome Centre for Human Genetics, University of Oxford, Oxford, United Kingdom,* [26]*Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA,* [27]*Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA,* [28]*Texas Children's Hospital, Houston, TX, USA,* [29]*Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX, USA,* [30]*Division of Genetics and Genomics, Boston Children's Hospital, Boston, MA, USA,* [31]*Section of Pediatric Neurology and Developmental Neuroscience, Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA,* [32]*Facultad de Medicina, Clinica Alemana Universidad del Desarrollo, Santiago, Chile,* [33]*Department of Diagnostic and Interventional Radiology, Heidelberg University Hospital, Heidelberg, Germany,* [34]*Institute of Human Genetics, Friedrich-Alexander-Universität (FAU), Erlangen, Germany,* [35]*Department of Chemistry, Vanderbilt University, Nashville, TN, USA,* [36]*Institute for Drug Discovery, University of Leipzig Medical Center, Leipzig, Germany,* [37]*Department of Anatomy and Cell Biology, College of Medicine, Alfaisal University, Riyadh, Saudi Arabia.*

Bi-allelic *TECPR2* variants have been associated with a complex syndrome with features of both a neurodevelopmental and neurodegenerative disorder. Core clinical symptoms entail intellectual disability, muscular hypotonia, dysarthria, gait abnormalities, peripheral neuropathy and autonomic dysfunction. To date, mostly truncating variants have been reported. Two are founder variants in Bukharian and Ashkenazi Jewish populations.Through an international collaboration, we identified 17 individuals from 15 families with bi-allelic *TECPR2*-variants. Eight of 17 distinct variants in the cohort were missense variants. Analysis of the spatial distribution revealed linear clustering of these variants in the N- and C-terminal protein region, in line with higher restrain for missense variation as indicated by higher computational scores and depletion of homozygous missense variants. As TECPR2 has no published crystal structure, we established a pipeline to predict 3D protein models based on three different algorithms (GalaxyTBM, swissmodel, trRosetta). We identified one 7-bladed β-propeller in the WD40 domain and two β-propeller motifs in the TECPR-repeat containing region separated by an unstructured linker region. All missense variants affected conserved residues in β-propeller units without clear clustering, indicating structural protein changes and faster degradation as possible pathomechanism. These results can be used for classification of missense variants according to ACMG guidelines as PM1_Supporting.With the results of our Human Phenotype Ontology (HPO)-based phenotype curation, we present an advanced framework for *TECPR2* missense reporting. Based on these analyses, we will establish a *TECPR2*-disease focused website allowing automated variant classification, comparison of phenotypes and display of current surveillance recommendations to aid clinicians involved.

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P08.076.B Characterizing a *de novo TRIO* gene variant as a likely cause of autosomal dominant Intellectual developmental disorder type 63 with macrocephaly

Doga Ceren Tekguc[1], Gulten Tuncel[2], Sefik Karanlik[3], Nevrez Koreken[3], Sehime Gulsun Temel[4], ***Mahmut Cerkez Ergoren[5]***

[1]Dr. Burhan Nalbantoglu State Hospital, Department of Pediatrics, Nicosia, Cyprus, [2]Near East University, DESAM Institute, Nicosia, Cyprus, [3]Near East University Hospital, Department of Medical Genetic Diagnosis Laboratory, Nicosia, Cyprus, [4]Bursa Uludag University, Faculty of Medicine, Department of Medical Genetics, Bursa, Turkey, [5]Near East University, Faculty of Medicine, Department of Medical Genetics, Nicosia, Cyprus.

3-years-old female patient, born to non-consanguineous healthy parents was admitted to the laboratory with a possible pre-diagnosis of Kabuki Makeup Syndrome. In paediatric examination, Bayley Scales of Infant and Toddler Development - III was performed and global developmental delay was observed as there was a significantly increased need for support in the areas of language, cognitive and movement development. As she also had dysmorphic findings including macrocephaly and ulnar deviation of the wrist, genetic aetiology was considered. In cytogenetic and molecular genetic analysis, no chromosomal abnormalities as well as no variation in genes associated with Kabuki Makeup Syndrome (CHD7, EYA1, IRF6, KDM6A, KMT2D) and no copy number variations (CNV) clinically related to the described phenotype has been identified. However, a heterozygous variant of uncertain significance (VUS), in the *TRIO* gene c.3199_3203delinsGAGCC p.(Lys1067_Glu1068delinsGluAla) was detected. Pathogenic variants in the *TRIO* gene have mainly been associated with autosomal dominant mental retardation type 44 with microcephaly and also autosomal dominant Intellectual developmental disorder type 63 with macrocephaly. In this context, the genetic diagnosis of an autosomal dominant TRIO-related disorder is possible but further segregation analysis with samples from parents is required to confirm the variation as *de novo*. In conclusion, classification of VUS variants by molecular analysis supported by clinical and experimental data is of great importance for diagnosis and development of treatment options in rare genetic disorders. Here we present the c.3199_3203delinsGAGCC p.(Lys1067_Glu1068delinsGluAla) VUS variant as a likely cause of the phenotypic clinical traits observed in our patient.

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P08.078.D Expanding the spectrum of *WAC*-related intellectual disability: two novel variants and a patient with congenital heart disease

Rita Quental[1]*, Daniel Gonçalves[2], João Parente Freixo[3], Miguel Leão[1]*

[1]Medical Genetics Service, São João University Hospital Centre, Porto, Portugal, [2]Pediatrics Service, São João University Hospital Centre, Porto, Portugal, [3]CGPP-IBMC, University of Porto, Porto, Portugal.

Introduction: Heterozygous pathogenic variants in *WAC* gene cause a rare syndrome characterized by intellectual disability, behavioural abnormalities, facial dysmorphism, gastrointestinal dysfunction, and, in some individuals, visual problems, sleep disturbance and seizures. Chromosomal deletions at 10p11p12 encompassing *WAC* gene have been described in patients with a similar phenotype, although some present with other clinical manifestations namely cardiac defects. **Cases:** We report two patients with a syndromic neurodevelopmental disorder whose clinical exome revealed novel variants in *WAC* gene. Patient 1 is a 7-year-old girl with dysmorphic features including synophrys, flat nasal bridge, protruding ears, hirsutism, and brachydactyly. Additionally, she had developmental delay, microcephaly, hypotonia, and constipation. Most remarkable is the presence of congenital heart disease (aortic coarctation associated with atrial and ventricular septal defects), which required two cardiac surgeries. Patient 2 is a 22-year-old male with microcephaly, intellectual disability and aggressive behaviour. He had synophrys, pectus excavatum, history of patellar luxation and kyphoscoliosis. Patient 1 has the variant c.1407del p.(Ser470Valfs*15) in heterozygosity in *WAC*, while in Patient 2 the c.811C>T p.(Gln271*) variant was detected. Both were *de novo* and classified as likely pathogenic.

Discussion: We report two patients with novel *de novo WAC* variants, one of which presenting a complex congenital cardiopathy. Although cardiac defects are a common feature in patients with 10p11p12 deletions, here we show that single-nucleotide *WAC* pathogenic variants causing a syndromic form of intellectual disability can also be associated with congenital heart defects. Therefore, this report explores insights on the phenotypic and genotypic spectrum of this rare syndrome.

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P08.079.A Biallelic loss-of-function mutations in *WDR11* are associated with microcephaly and intellectual disability

Natja Haag[1], Ene C. Tan[2], Matthias Begemann[1], Lars Buschmann[1], Petra Hoschbach[3], Angeline H. M. Lai[2], Maggie Brett[2], Ganeshwaran H. Mochida[4,5], Stephanie DiTroia[6], Lynn Pais[6], Jennifer E. Nail[4,5,7], Muna Al-Saffar[4,5,8], Laila Bastaki[9], Christopher A. Walsh[4,5,7], Ingo Kurth[1], ***Cordula Knopp[1]***

[1]Institute of Human Genetics, Medical Faculty, RWTH Aachen University, Aachen, Germany, [2]KK Research Centre/Genetics Service, KK Women's & Children's Hospital, Singapore, Singapore, [3]Division of Neuropediatrics and Social Pediatrics, Department of Pediatrics, Medical Faculty, RWTH Aachen University, Aachen, Germany, [4]Division of Genetics and Genomics, Boston Children's Hospital, Boston, MA, USA, [5]Departments of Pediatrics and Neurology, Harvard Medical School, Boston, MA, USA, [6]Center for Mendelian Genomics, Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, MA, USA, [7]Howard Hughes Medical

Institute, Boston Children's Hospital, Boston, MA, USA, [8]Department of Paediatrics, College of Medical and Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirates, [9]Kuwait Medical Genetics Centre, Maternity Hospital, Shuwaikh, Kuwait.

Introduction: In humans, heterozygous missense variants in the *WDR11* gene have been associated with hypogonadotropic hypogonadism. In contrast, *Wdr11*-null mice and knockdown-zebrafish demonstrated complex developmental abnormalities in multiple organs resembling features known to be associated with hedgehog signaling and ciliogenesis. However, no human phenotype associated with biallelic variants in *WDR11* has been described yet.

Material and Methods: Whole exome sequencing was performed in six affected individuals from three unrelated families. Independent discoveries were shared through GeneMatcher. All patients underwent comprehensive clinical examination. WDR11 protein expression was studied in patient-derived fibroblasts using western blot (WB) analysis and immunohistochemical (IHC) staining.

Results: Biallelic loss-of-function mutations in *WDR11* were identified in six individuals from three independent families. Affected patients show a distinct phenotype that includes microcephaly, mild short stature and intellectual disability of variable degree. Complete loss of *WDR11* protein in fibroblasts was demonstrated by WB and IHC analyses for one affected patient. Heterozygous carriers of *WDR11* loss-of-function variants in our families were healthy and did in particular not show any obvious clinical signs of hypogonadotropic hypogonadism as seen in patients with heterozygous missense variants.

Conclusions: Our findings suggest that biallelic *WDR11* variants in humans result in an overlapping but milder phenotype compared to *Wdr11*-deficient animals. However, the observed human phenotype differs significantly from dominantly inherited mutations leading to hypogonadotropic hypogonadism, suggesting that recessive *WDR11* mutations define a distinct clinically entity.

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P08.080.B Skraban-Deardorff Syndrome: six new cases of *WDR26*-related disease and expansion of the clinical phenotype

Auriane Cospain[1], *Elise Schaefer*[2], *Marie Faoucher*[3,4], *Christèle Dubourg*[3,4], *Wilfrid Carré*[3,4], *Varoona Bizaoui*[5], *Jessica Assoumani*[6], *Lionel Van Maldergem*[6], *Amélie Piton*[7], *Bénédicte Gérard*[7], *Frédéric Tran Mau-Them*[8,9], *Ange-Line Bruel*[8,9], *Laurence Faivre*[8,9], *Florence Demurger*[10], *Laurent Pasquier*[11], *Sylvie Odent*[1,4], *Mélanie Fradin*[1], *Alinoë Lavillaureix*[1,4]

[1]*CHU Rennes, Service de Génétique Clinique, Centre de Référence Maladies Rares CLAD-Ouest, ERN ITHACA, Hôpital Sud, Rennes, France,* [2]*Service de Génétique Médicale, Institut de Génétique Médicale d'Alsace, Strasbourg, France,* [3]*Service de Génétique Moléculaire et Génomique, CHU, Rennes, Rennes, France,* [4]*Univ Rennes, CNRS, IGDR, UMR 6290, Rennes, France,* [5]*Service de Génétique, Centre Hospitalier Universitaire de Caen Normandie, Caen, France,* [6]*Centre de Génétique Humaine, Université de Franche-Comté, Besançon, France,* [7]*Laboratoire de Diagnostic Génétique, Institut de Génétique Médicale d'Alsace, Hôpitaux Universitaires de Strasbourg, Strasbourg, France,* [8]*Centre de Référence Anomalies du développement et syndromes malformatifs, Fédération Hospitalo-Universitaire Médecine Translationnelle et Anomalies du Développement (TRANSLAD), CHU de Dijon Bourgogne, Dijon, France,* [9]*Inserm UMR1231 GAD, Génétique des Anomalies du Développement, Université de Bourgogne, Dijon, France,* [10]*Service de Pédiatrie Génétique, CH de Vannes, Vannes, France,* [11]*Service de Génétique Clinique, Centre Référence "Déficiences Intellectuelles de causes rares" (CRDI), Centre Hospitalier Universitaire de Rennes, Rennes, France.*

Introduction: Skraban-Deardorff syndrome (a disease related to variations in the *WDR26* gene; OMIM #617616) was first described in a cohort of 15 individuals in 2017, no other cases have been described since. Here, we report on six novel cases with heterozygous *de novo WDR26* pathogenic variants. We provide additional clinical and molecular data and compare the probands' phenotypes with the literature data.

Materials and methods: Clinical and molecular data were collected from six patients treated at four different university hospitals in France. *WDR26* variants were detected with next-generation sequencing (whole exome sequencing or screening against an intellectual deficiency and epilepsy gene panel).

Results: We observed intellectual disability, developmental delay (predominantly for the language), early-onset epilepsy, skeletal manifestations, abnormal gait, characteristic dysmorphism, and a happy/friendly personality consistent with the original description. One patient displayed marked hypotonia with Z-line disorganisation and multiminicores on the muscle biopsy. Four patients had intrauterine growth restriction. Five patients had feeding difficulties during infancy; one had a suspected Pierre-Robin sequence, another had a velar cleft palate. Our patients with *WDR26*-related syndrome had a pronounced subpalpebral crease, rounded palpebral fissures and relatively large irises, not described previously. Language appears to be limited to single words or two-word associations.

Conclusion: We confirm the rich gastrointestinal and musculoskeletal morbidity and velar cleft as part of the clinical variability of this condition. Early speech therapy is crucial in order to improve language and oral eating and drinking. We additionally observed abnormal features in a muscle biopsy; this finding warrants further investigation.

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P08.082.D Importance of a multidisciplinary team when analyzing WES: higher diagnostic rate, increased confidence and better care

Sérgio B. Sousa[1,2], *Maria José Simões*[3,4], *Belinda Campos-Xavier*[1], *Hugo Froufe*[3,4], *Carolina Ribeiro*[2], *João Mendes*[2], *Joaquim Sá*[1], *Margarida Venâncio*[1,2], *Conceição Egas*[4,5], *Ana Catarina Gomes*[3], *Jorge M. Saraiva*[1,6]

[1]*Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal,* [2]*Medical Genetics Institute - UC Genomics, Faculty of Medicine, University of Coimbra, Coimbra, Portugal,* [3]*CBR Genomics, Cantanhede, Portugal,* [4]*Genoinseq, Next-Generation Sequencing Unit, Biocant, Cantanhede, Portugal,* [5]*Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal,* [6]*University Clinic of Pediatrics, Faculty of Medicine, Universidade de Coimbra, Coimbra, Portugal.*

The diagnostic rate of whole exome sequencing (WES) is quite variable, being the main challenge the accurate interpretation of

variants for which the dialogue laboratory-clinicians is fundamental. Herein, we present the results of the In2Genome Project whose main goal was to develop a multidisciplinary approach to the WES-based genetic diagnosis by having both medical and laboratorial geneticists working directly together in each case. We analyzed a group of 62 undiagnosed patients with intellectual disability (ID) syndromes. A sequential approached was used in most cases: first, single WES was performed, being the analysis based on an ID virtual gene panel; second, in the inconclusive cases (n = 42) the parents were sequenced, and trio analysis undertaken.

The diagnostic rate for the single analyses was 29.1% and increased, after trio analyses, to 45.2% (28/62 of overall rate). Thirty causal variants, 22 of which novel, were identified in 28 genes. The advantages of this multidisciplinary approach were clear: not only the higher diagnostic rate (significantly above most reported studies and comparing to our experience when WES was analyzed in an independent laboratory), but also the more cost-effectiveness and certainly the increased team satisfaction and confidence in the results. Additionally, there is a much easier translation to a research setting, data reanalysis and collaboration with other centers in the remaining unsolved cases (additional 3 candidate genes so far). The In2Genome project CENTRO-01-0247-FEDER-017800 was supported by Centro Portugal Regional Operational Programme (CENTRO 2020), under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (ERDF).

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P08.083.A A WES study in 200 intellectual disability/ autism patients

Lucia Pia Bruno[1,2], *Gabriella Doddato*[1,2], *Floriana Valentino*[1,2], *Annarita Giliberti*[1,2], *Caterina Lo Rizzo*[3], *Maria Antonietta Mencarelli*[3], *Francesca Mari*[1,2,3], *Anna Maria Pinto*[3], *Francesca Fava*[1,2,3], *Margherita Baldassarri*[1,2], *Andrea Tommasi*[1,2,3], *Alessandra Fabbiani*[1,2,3], *Vittoria Lamacchia*[1,2,3], *Elisa Benetti*[2], *Simone Furini*[2], *Francesca Ariani*[1,2,3], *Alessandra Renieri*[1,2,3], *Chiara Fallerini*[1,2]

[1]*Medical Genetics, University of Siena, Siena, Italy,* [2]*Med Biotech Hub and Competence Center, Department of Medical Biotechnologies, University of Siena, Siena, Italy,* [3]*Genetica Medica, Azienda Ospedaliera Universitaria Senese, Siena, Italy.*

Intellectual disability (ID) is a disorder characterized by an incomplete or arrested mental development and by IQ less than 70. Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by social impairment, restricted interests and repetitive behaviors. ID and ASD symptoms are often overlapping. In the present study, we investigated by Whole Exome Sequencing (WES) analysis a total of 200 ID/ASD patients. Our cohort included 40 patients with syndromic ID, 64 with non-syndromic ID, 6 with autism and syndromic ID, 19 with autism and non-syndromic ID, and71 with isolated autism. We identified 39 patients with pathogenic variants (PVs) with a detection rate of 20%. In particular, 29 PVs were found in ID patients and 3 in ASD patients. 7 PVs were identified in patients with ID and ASD features. Regarding variant type, 13 variants were missense changes accounting for 33% of the total, 7 were frameshift, 14 were nonsense, 4 were splicing changes and 1 was inframe deletion. We report more de novo variants (37) than inherited ones (14). The majority of the mutated genes belongs to 4 biological pathways or regulate them: RAS/MAPK (e.g. FGFR3, PTPN11), Wnt/ß-catenin (e.g. DDX3X,GRIN2A, GRIN1), Sonic Hedgehog (e.g. B9D1,C2CD3) and GPCR signaling (e.g. DDX3X, MKS1). In conclusion, our results demonstrated the efficiency of WES analysis on the identification of PVs in ID/ASD patients. Moreover this study allowed to subdivide the causing genes into four groups according to the biological pathways suggesting new molecular interactions.

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P08.084.B WES result reanalysis and CAMK2B variant causing developmental delay

Todor Arsov[1,2], *Aspazija Sofijanova*[3], *Marcin Adamski*[2], *Carola Vinuesa*[2]

[1]*Ss. Cyril and Methodius University in Skopje, Faculty of Medicine-Skopje, Institute of Immunobiology and Human Genetics, Skopje, Macedonia, The Former Yugoslav Republic of,* [2]*Australian National University, John Curtin School of Medical Research, Centre for Personalized Immunology, Canberra, Australia,* [3]*Ss. Cyril and Methodius University in Skopje, Faculty of Medicine-Skopje, PHI University Clinic for child diseases, Skopje, Macedonia, The Former Yugoslav Republic of.*

CAMK2B has recently been found to cause Intellectual Disability Autosomal Dominant 54 (MRD54, OMIM 617799), characterized with global developmental delay, hypotonia, difficulty holding the head, delayed walking (or inability to walk) and speech (50% of patients are averbal), behavioral problems, intellectual disability, visual impairment, gastrointestinal problems and facial dysmorphic features. Some patients have seizures with EEG changes and brain imaging is generally normal. We describe a 3-year old patient with global developmental delay recognized at an early age, characterized with hypotonia and inability to hold the head, lack of speech, inability to walk, seizures (controlled on anti-epileptic drugs) and behavioral problems. He is a second child from a third pregnancy of non-consanguineous parents with history of anencephaly in the first pregnancy and a healthy daughter. The parents embarked on a long and expensive diagnostic odyssey, including CMA and trio WES testing returning no result. WES data re-analysis a year later identified a "de novo" pathogenic variant in CAMK2B (NM_172079.2:c.709G>A), not present in the databases of human variation, predicted damaging by "in silico" tools, affecting conserved amino acid residue and described previously in one other patient with intellectual disability of different ethnic origin. The result helped end the diagnostic odyssey in this family, provided reassurance in terms of the recurrence risk, and addressed some longstanding misconceptions about a "likely X-linked condition/inheritance pattern" in the family. This case illustrates the diagnostic utility of WES data re-analysis and importance of periodically revisiting uninformative results against growing evidence base for genetic causes of disease.

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P08.085.C Whole-exome sequencing as an effective tool for the detection of DNA sequence and structural variants in the pathogenesis of neurodevelopmental disorders

Marketa Wayhelova[1,2], *Vladimira Vallova*[3,2], *Jan Smetana*[1], *Petr Broz*[1], *Aneta Mikulasova*[4], *Dominika Loubalova*[1], *Renata Gaillyova*[5], *Petr Kuglik*[1,2]

[1]*Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic,* [2]*Centre of Molecular Biology and Genetics, University Hospital Brno, Brno, Czech Republic,* [3]*Deaprtment of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic,* [4]*Biosciences Institute, Newcastle University, Newcastle upon Tyne, United Kingdom,* [5]*Department of Medical Genetics and Genomics, University Hospital Brno, Brno, Czech Republic.*

With more than 50% diagnostic yield the whole-exome sequencing (WES) represents an effective and powerful tool to identify causes of neurodevelopmental disorders (NDDs) at the molecular level. We present our experience with WES as an effective tool for the detection of pathogenic sequence variants, copy-number variations (CNVs) and losses of heterozygosity (LOH) using the commercial kit Human Core Exome (Twist Biosciences) and Illumina NovaSeq 600. Our pilot study included 20 families (trios or quatros) of children with severe NDDs and associated congenital abnormalities. In the optimization step for CNV detection using read-depth approach we confirmed and specified all CNVs and LOH regions previously detected by array-CGH+SNP in 8 families. Mainly, we identified recurrent *de novo* pathogenic sequence variants in clinically relevant *SHANK3, GRIN1* and *NSD1* genes, novel *de novo* pathogenic variants in *KDM1A, KMT2E* and *GNAI1* genes, and a pathogenic sequence variant in *EDA* gene of maternal origin. All clinically relevant findings were manually verified using Sanger sequencing and qPCR and interpreted using a multistep approach based on information in integrated databases of genomic variants, relevant scientific literature, and individual anamnesis. Our pilot results confirm WES as a first-tier diagnostic test in the genetic evaluation of children with NDDs. Supported by Ministry of Health of the Czech Republic, grant nr. NU20-07-00145. All rights reserved.

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P08.086.D Xia-Gibbs syndrome - variable clinical manifestation of three cases from a single genetic department

Anna Kutkowska-Kaźmierczak*, Paweł Własienko, Maria Boczar, Olga Malinowska, Tomasz Gambin, Małgorzata Kruk, Agata Lipiec, Jerzy Bal, Ewa Obersztyn, Monika Gos*

Institute of the Mother and Child, Warsaw, Poland.

Introduction: Xia-Gibbs syndrome (XGS) is a rare neurodevelopmental disorder characterized by developmental delay, behavioral problems, speech delay, hypotonia and seizures. Most of the patients with XGS have a heterozygous loss-of-function *AHDC1* mutations.

Patients and methods: We present clinical evaluations of 3 patients with the clinical features of XGS, aged from 5 to 10 years. The mutation analysis was performed using targeted next generation sequencing.

Results: All patients had intellectual disability from mild to severe, delayed or absent speech, hypotonia, motor development delay, unstable gait, open mouth appearance, drooling, stereotypic movements of hands, characteristic pattern of behavior with aggression, auto-aggression and tantrums. Two patients had macrocephaly, one of them - delayed closure of fontanel and one patient - abnormal skull shape. Hypoplastic corpus callosum and simian crease were found in 2 individuals. One patient presented short stature treated successfully with growth hormone. One patient had accessory nipples and 2 whirls of hair. Molecular analysis revealed the presence of pathogenic variants c.917del (p.Pro306Leufs*146) and c.976_988del (p.Ser326Thrfs*122) in *AHDC1* gene in 2 patients. In one individual, variant of unknown significance c.1037G>A (p.Arg346His) was found.

Conclusions: The presence of macrocephaly, abnormal skull shape and delayed fontanel closure as well as short stature in our patients with XGS confirms the role of *AHDC1* gene product in skeleton development, especially in skull formation and progressive growth. The studies were supported from Institute of Mother and Child intramural grant no. OPK-510-18-63.

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P08.087.A A new neurodevelopmental disease with brain abnormalities due to *YWHAE* loss-of-function variants: from human to mice

Anne-Sophie Denommé-Pichon[1,2,3], *Ange-Line Bruel*[1,2], *Stephan C. Collins*[2], *Anna Mikhaleva*[4], *Christel Wagner*[5], *Valerie E. Vancollie*[6], *Mathys Weber*[2,7], *Carlos Prada*[8], *Alexis Overs*[1,2], *María Palomares-Bralo*[9,10,11], *Fernando Santos-Simarro*[9,10,11], *Marta Pacio-Míguez*[11], *Tiffany Busa*[12], *Eric Legius*[13], *Carlos A. Bacino*[14], *Jill A. Rosenfeld*[15], *Maria Antonietta Mencarelli*[16], *Ilaria Longo*[16], *Alessandra Renieri*[16,17,18], *Salvatore Grosso*[19,20], *Frédéric Tran Mau-Them*[1,2], *Antonio Vitobello*[1,2,3], *Yannis Duffourd*[1,2], *Christopher J. Lelliott*[6], *Christel Thauvin-Robinet*[1,2,21], *Christophe Philippe*[1,2], *Binnaz Yalcin*[2], *Laurence Faivre*[2,3,7]

[1]*Unité Fonctionnelle Innovation en Diagnostic génomique des maladies rares, FHU-TRANSLAD, CHU Dijon Bourgogne, Dijon, France,* [2]*UMR1231 GAD, Inserm - Université Bourgogne-Franche Comté, Dijon, France,* [3]*European Reference Network, ERN ITHACA, Dijon, France,* [4]*Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland,* [5]*IGBMC, UMR7104, Inserm, U964, Illkirsch, France,* [6]*Wellcome Sanger Institute, Hinxton, Cambridge, United Kingdom,* [7]*Centre de Référence Maladies Rares « Anomalies du développement et syndromes malformatifs », Centre de Génétique, FHU-TRANSLAD et Institut GIMI, CHU Dijon Bourgogne, Dijon, France,* [8]*Cincinnati Children's Hospital Medical Center, University of Cincinnati School of Medicine, Cincinnati, OH, USA,* [9]*Instituto de Genética Médica y Molecular (INGEMM), Hospital Universitario La Paz, Universidad Autónoma de Madrid, IdiPAZ, Madrid, Spain,* [10]*Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER, U753), Instituto Carlos III, Madrid, Spain,* [11]*European Reference Network, ERN ITHACA, Madrid, Spain,* [12]*Département de génétique médicale, CHU Timone enfants, AP-HM, Marseille, France,* [13]*Laboratory for Neurofibromatosis Research, Department of Human Genetics, KU Leuven University Hospital, Leuven, Belgium,* [14]*Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA,* [15]*Baylor Genetics Laboratories, Houston, TX, USA,* [16]*Genetica Medica, Azienda Ospedaliera Universitaria Senese, Siena, Italy,* [17]*Medical Genetics, University of Siena, Siena, Italy,* [18]*Med Biotech Hub and Competence Center, Department of Medical Biotechnologies, University of Siena, Siena, Italy,* [19]*Dipartimento di Medicina Molecolare e dello Sviluppo, Universita' degli Studi di Siena, Siena, Italy,* [20]*U.O.C. Pediatria, Azienda Ospedaliera Universitaria Senese, Siena, Italy,* [21]*Centre de Référence Déficiences Intellectuelles de Causes Rares, FHU-TRANSLAD, CHU Dijon Bourgogne, Dijon, France.*

Introduction: Miller-Dieker syndrome is caused by a contiguous gene deletion syndrome involving multiple genes on chromosome 17p13.3, especially *PAFAH1B1* and *YWHAE*. Toyo-oka *et al*.

showed that deletions comprising *YWHAE* were responsible for the most severe Miller-Dieker cases. Since then, several patients with 17q13.3 deletions involving *YWHAE* have been reported and presented with developmental delay and cerebral abnormalities. To date, no *YWHAE* loss-of-function single nucleotide variants (SNV) has been described, and the gene has not been reported as clearly morbid yet.

Materials and Methods: Three patients with *YWHAE de novo* heterozygous loss-of-function SNVs and 5 patients (3 unpublished) with deletions (<1 Mb) encompassing *YWHAE* were gathered through different data-sharing networks (GeneMatcher, Decipher, AnDDI-Rares and ITHACA). To address the specific impact of *YWHAE* loss of function in the neurodevelopmental phenotype of Miller-Dieker syndrome, we generated a full knockout of *Ywhae* in the mouse and assessed neuroanatomical parameters in conjunction with 3D brain imaging techniques in mouse embryos and adults.

Results. The most frequent manifestations in patients were developmental delay, delayed speech, seizures and brain malformations (corpus callosum hypoplasia, delayed myelination, ventricular dilatation). Patients with SNVs have no dysmorphic features whereas those with large deletions presented with facial features. Studies in mouse *Ywhae*$^{-/-}$ revealed craniofacial characteristics and numerous brain structural defects (thin cortices, corpus callosum dysgenesis and hydrocephalus) paralleling those seen in the human condition.

Conclusion: These studies confirm *YWHAE* loss-of-function variants as cause of a new rare neurodevelopmental disease associated with brain abnormalities in human and mouse.

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P09 Neurogenetic and Psychiatric Disorders

P09.001.B 17p13.3 microduplication syndrome: new cases with class I and class II gains and clinical and molecular delineation of the syndrome

Julián Nevado[1,2,3], *Jair A. Tenorio*[1,2,3], *Ameni Touati*[1], *Jesús Pozo-Román*[4], *Mabel Segovia*[5], *Pedro Arias*[1], *Natalia Gallego*[1], *Gema Gordo*[1], *Irene Dapia*[1], *SOGRI Consortium, Pablo D. Lapunzina*[1,2,3]

[1]*INGEMM-IdiPaz, Madrid, Spain,* [2]*CIBERER, Madrid, Spain,* [3]*ITHACA European Research Network, Madrid, Spain,* [4]*Hospital Universitario Niño Jesús, Madrid, Spain,* [5]*CENAGEM, Buenos Aires, Argentina.*

Chromosome 17p13.3 is a region of genomic instability due to extensive LCRs which make it vulnerable to copy number variations. Depending on whether a deletion or a duplication of 17p13.3 occurs, different rare neurodevelopmental disorders arise. Phenotypic features of 17p13.3 microduplication-syndrome (MIM #613275) include developmental and psychomotor delay, behavioral problems, distinct physical features, postnatal-overgrowth and ASD, as well as limb malformations and cleft lip and palate. Genes thorough this region; *CRK, PAFAH1B1*, and *YWHAE* have crucial roles in neuronal migration and contribute to each of these genetic disorders. *BHLAH9* located within chromosome 17p13.3, but immediately outside of the Miller-Dieker Syndrome critical region, seems to be necessary but not sufficient, for limb malformation. Depending on the genes involved, patients with duplications in this region may be categorized into either class I or class II. Individuals in class I have microduplications of the *YWHAE*, but not *PAFAH1B1* and generally, result in learning disabilities, autism, and developmental delays. Individuals in class II always have microduplications of the *PAFAH1B1* gene, which may include *YWHAE* and other genetic gains. Class II-microduplications generally result in smaller body size, developmental delays, microcephaly, and other brain malformations. We review the phenotypes associated with copy number gains of chromosome 17p13.3 in several cases of Class I and Class II-microduplications observed in patients from The Spanish Overgrowth Registry Initiative (SOGRI) by means SNP-arrays, in which the targeted analysis was negative or in which the clinical features were not compatible with any of the well-known Overgrowth disorders.

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P09.002.C Mouse neuronal cells outperform in a novel galactonamide molecular gel rather than in neurosphere; comparison of neurogenesis and neuronal differentiation mRNA markers

Keziban Korkmaz Bayram[1], *Juliette Fitremann*[2], *Arslan Bayram*[3], *Zeynep Yılmaz*[4], *Ecmel Mehmetbeyoğlu*[5], *Yusuf Özkul*[5], *Minoo Rassoulzadegan*[6]

[1]*Medical Genetics Department, Ankara Yildirim Beyazit University, Ankara, Turkey,* [2]*Université de Toulouse, Toulouse, France,* [3]*Medical Genetics Department, Etlik Zubeyde Hanım Women's Diseases Education and Research Hospital, Ankara, Turkey,* [4]*Genome and Stem Cell Center (GENKOK), Erciyes University, Kayserı, Turkey,* [5]*Genome and Stem Cell Center (GENKOK), Erciyes University, Kayseri, Turkey,* [6]*University of Nice Sophia Antipolis, Nice, France.*

N-heptyl-D-galactonamide (GalC7) is a synthetic carbohydrate derivative that self-assemble into supramolecular fibers - biocompatible 3D hydrogels. In this hydrogel, neural stem cells differentiate into both glial cells and neurons. In this study, the gene expression of mouse hippocampal stem cells has been investigated in several conditions. The analysis was carried out either directly ex vivo on the cells of the fresh hippocampus or after culture of the primary cells in vitro under three conditions: (1) culture in neurospheres in non-adherent conditions for 19 days, (2) direct culture in GalC7 for 7 days and (3) culture in GalC7 for 7 days after 12 days in neurospheres; to complete culture days to 19 as in (1). *Sox8* and *Sox10*, oligodendrocyte markers, and *Sox9*, an astrocyte marker, were expressed at a much higher level on GalC7. *Dcx*, a marker of neurogenesis and *Neurod1*, a marker of neuronal differentiation, are expressed at a very low level compared to the fresh hippocampi conditions both in neurospheres and in GalC7 hydrogels. However, these two markers were maintained at better levels in the GalC7 gel culture compared to the neurosphere condition. These results show that the GalC7 hydrogel brings different and interesting conditions for inducing the differentiation and maturation of neural progenitor cells compared with polymer-based scaffolds or cell-only conditions. Microstructure of the fibrous network, the chemical composition, and the bioavailability of the gelling molecule make cell culture in supramolecular hydrogels very different. The differences observed open new perspectives in tissue engineering, induction, and gene expression analysis.

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P09.003.D Transcriptomic characterization of 7q11.23 patient-specific induced pluripotent stem cells (iPSCs) and derivates

Mar Costa-Roger[1], *Bernd Kuebler*[2], *Marina Alvarez-Estape*[1], *Raquel Flores*[1], *Luis Alberto Pérez-Jurado*[1,3], *Ivon Cuscó*[1,4], *Roser Corominas*[1,5]

[1]*Genetics Unit, Departament de Ciències Experimentals i de la Salut, Pompeu Fabra University, Hospital del Mar Research Institute (IMIM), Parc de Salut Mar and Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Barcelona, Spain,* [2]*Regenerative Medicine Programme, Institut d'Investigació Biomèdica de Bellvitge, IDIBELL, L'Hospital de Llobregat, and Spanish National Stem Cell Bank-Barcelona Node, Instituto de Salud Carlos III (ISCIII), Madrid, Spain,* [3]*Genetics Service, Parc de Salut Mar, Barcelona, Spain,* [4]*Department of Clinical and Molecular Genetics and Medicine Genetics Group, VHIR, University Hospital Vall d'Hebron, Barcelona, Spain,* [5]*Department of Genetics, Microbiology and Statistics, Barcelona University, Barcelona, Spain.*

Introduction: Williams-Beuren syndrome (WBS;OMIM#194050) and 7q11.23 microduplication syndrome (DUP7;OMIM#609757) are rare multisystem disorders with somehow opposed neurobehavioral trajectories caused by 1.55-1.84Mb heterozygous microdeletion or microduplication of 26-28 contiguous genes at 7q11.23, respectively. Cellular reprogramming is a good approach to overcome the experimental limitations to study neurodevelopmental disorders in humans. The purpose of this project is to evaluate the transcriptomic consequences of 7q11.23 patient-derived iPSC lines and derivatives.

Methods: We generated patient-specific iPSCs from fibroblasts from four WBS patients, four DUP7 patients and two controls, which were differentiated to neural progenitor cells (NPCs) and to dopaminergic neurons. After RNA extraction from all cell-types (fibroblasts, iPSCs, NPCs and neurons), we assessed genome-wide differential expression using expression microarrays.

Results: We identified a total of 722 differentially expressed genes (DEGs) in a pairwise comparison of the three genotypes in all cell-types. Half of the genes in 7q11.23 showed mirroring expression between DUP7 and WBS models. Enrichment analysis of DEGs in fibroblasts revealed specific pathways and gene ontology categories relevant for the hallmark phenotypes of both disorders. Neuronal processes, such as transmission across chemical synapses, were significantly enriched in neurons. In addition, WGCNA of iPSCs and NPCs lines uncovered interesting co-expression modules related to ion transport (q-value = 0.0365) or glutamate receptor signaling (q-value = 0.0463), respectively.

Conclusions: Integrative transcriptomic analysis of in vitro 7q11.23-CNVs cellular models reveals genes and pathways altered during early neuronal development in these genomic disorders, which could lead to novel potential therapeutic targets. Funded by grants **FPU16/06907**, **FIS16/00369**, **H2020-MSC-656359**, **RYC-2017-21636**, **RTI2018-101960-A-I00**, **CIVP16A1828**, **RD12/0019/0034**, **PRB2;PT13/0001/0041**.

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P09.004.A Relevance of double-hit mechanisms in patients with Neurodevelopmental Disorders (NDDs): a re-evaluation of 526 patients with non-benign copy number variants (CNVs)

Martina Servetti[1,2], *Livia Pisciotta*[1,3], *Elisa Tassano*[4], *Lino Nobili*[1,5], *Silvia Boeri*[1,5], *Maria Cerminara*[1], *Margherita Lerone*[2], *Maria Teresa Divizia*[2], *Patrizia Ronchetto*[4], *Aldamaria Puliti*[1,2]

[1]*Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health (DiNOGMI), University of Genova, Genova, Italy,* [2]*Medical Genetics Unit, IRCCS Istituto Giannina Gaslini, Genova, Italy,* [3]*Child Neuropsychiatry Unit, ASST Fatebenefratelli Sacco, Milano, Italy,* [4]*Human Genetics Laboratory, IRCCS Istituto Giannina Gaslini, Genova, Italy,* [5]*Child Neuropsychiatry Unit, IRCCS Istituto Giannina Gaslini, Genova, Italy.*

Introduction: Inconsistency of genotype-phenotype correlations often complicates the clinical interpretation of CNVs. Increasing findings suggest that such inconsistency may be also explained by complex interactions among multiple CNVs. The aim of this study was to re-evaluate patients with CNVs previously classified as variant of uncertain significance (VOUS) to unveil new candidate genes and pathogenetic mechanisms that could explain the patient's phenotype.

Methods: CNVs identified by diagnostic array-CGH in 526 NDD cases were re-analysed. A deep analysis, mainly consisting in a revision of gene expression/function annotation, and chromatine organization data, was performed. New candidate genes were analysed by GeneCodis4 to evidence enrichment for known NDD-associated GeneOntology terms and pathways.

Results: In 42% of cases pathogenic CNVs were found, while in 58% identified CNVs remained initially VOUS. Notably, 3.5% of patients with variants classified as VOUS had two CNVs, each inherited from one of the two healthy parents and overlapping known and/or new candidate NDD genes, that could act by double-hit mechanisms. Interestingly, two deletions involving two new candidate genes, *PTPRD* and *BUD13*, were found in a patient with neuropsychiatric and neurological features that together could fully explain the patient's phenotype. Double-hit mechanisms were also found among the 225 patients with pathogenic CNVs, as those with syndromic CNVs (9%) and with inherited CNVs encompassing known NDD genes (14%), that could account for variable expressivity and incomplete penetrance.

Conclusions: In our cohort of patients CNV-mediated double-hit mechanisms seem to play a relevant role in NDDs, helping to elucidate complex phenotypes.

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P09.005.B Sex specific effect of *ATXN2* rs7969300 polymorphism on age of onset in Spinocerebellar Ataxia type 2

Luis E. Almaguer-Mederos[1], *Suzana Gispert*[2], *Dennis Almaguer-Gotay*[1], *Raúl Aguilera-Rodríguez*[1], *Yanetza González-Zaldívar*[1], *Yaimé Vázquez-Mojena*[1], *Dany Cuello-Almarales*[1], *Daniel O. Palenzuela*[3], *Georg Auburger*[2]

[1]*Center for the Investigation and Rehabilitation of Hereditary Ataxias, Holguín, Cuba,* [2]*Goethe University Medical Faculty, Frankfurt, Germany,* [3]*Center of Genetic Engineering and Biotechnology, Havana, Cuba.*

Background: Spinocerebellar ataxia type 2 (SCA2) shows huge clinical variability even in individuals sharing the same CAG repeat length. A large study was conducted to assess the role of SNP rs7969300 as a modifier of the age of onset in SCA2 patients.

Methods: A cross-sectional study involving 427 Cuban clinically and molecularly confirmed SCA2 patients was conducted. The

ATXN2 CAG-repeat length was determined by PCR followed by polyacrylamide gel electrophoresis, while the SNP rs7969300 was assessed by qPCR using a TaqMan assay.

Results: The mutant "T" allele for SNP rs7969300 was detected in 17 out of 427 individuals, for a frequency of 0.0398. Regression analysis showed that SNP rs7969300 genotypes contributed in a 0.3% to explain age of onset variability ($p = 0.029$), while the interaction term between SNP rs7969300 genotypes and sex contributed in a 0.5% ($p = 0.012$). The occurrence of one "T" allele produced an average age of onset of 6.41 years earlier than expected in male SCA2 patients.

Conclusions: Evidence for a sex-specific effect of SNP rs7969300 on the age of onset of SCA2 patients is provided. Further replication studies in different SCA2 populations in the world will be needed to clarify the role of this SNP on the SCA2 clinical phenotype. Also, further functional studies would be valuable to establish the role of SNP rs7969300 in SCA2 physiopathology.

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P09.007.D An apparently *de novo* Alexander-associated *GFAP* mutation transmitted from a healthy mother showing gonosomal mosaicism

Alice Grossi, *Tiziana Bachetti, Isabella Ceccherini*

Istituto Giannina Gaslini, Genova, Italy.

Alexander disease (AxD) is a leukodystrophy caused by heterozygous mutation in the *GFAP* gene, presenting at different age of onset: early childhood (type I) and later, up to adult (type II). To date, pathogenic variants identified throughout the gene explain more than 90% of the patients. Rare recurrence of AxD in siblings, without apparent parental transmission, allowed to hypothesize the occurrence of germinal and eventually somatic mosaicism, a circumstance however never proven so far.Next Generation Sequencing (NGS) with deep coverage (≥500X) at the *GFAP* locus, of DNA isolated from peripheral blood samples, was performed in 11 probands, carrying apparently *denovo* GFAP mutations, and their healthy parents. One mother revealed a mosaicism of the GFAP mutation already detected in her son. The ratio between coverages of mutant and wildtype alleles, compared to those obtained with known dilutions of the mutant allele, provided an estimate of the mosaicism extension (12.18%) (**A**). This result was confirmed through single nucleotide primer extension and ratio of clones carrying mutant and wildtype inserts, obtaining 8.9% and 10%, respectively.Though the search for parental gonosomal mosaicisms should make use, whenever possible, of the relevant tissues, deep NGS of even less appropriate DNA sources may represent a fruitful approach to this type of investigation. The genetic counseling to AxD families should always take this rare event into account.

A

	180A_50%	180A_25%	180A_12,5%	180A_6,125%	110M_ unknown
TOT (X)	720	656	739	760	**673**
WT (X)	**371**	**549**	**661**	**730**	**591**
MUT (X)	**349**	**107**	**78**	**30**	**82**
%	48,47	16,31	10,55	3,95	**12,18**

A. Grossi: None. **T. Bachetti:** None. **I. Ceccherini:** None.

P09.008.A Alkuraya-Kucinskas syndrome: novel compound heterozygous *KIAA1109* variants in two siblings with this recently delineated disorder of brain development

Matina Prapa[1], *Suzanna Dunkerton*[2], *Jeremy Brockelsby*[2], *Nishigandh Deole*[3], *Pooja Harijan*[4], *Marion Bohatschek*[5], *Elspeth Whitby*[6], *Joan Paterson*[1], *Soo-Mi Park*[1], *Sarju G. Mehta*[1]

[1]*East Anglian Medical Genetics Service, Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom,* [2]*Department of Obstetrics and Gynaecology, Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom,* [3]*Department of Obstetrics and Gynaecology, East Suffolk and North Essex NHS Foundation Trust, Ipswich, United Kingdom,* [4]*Department of Paediatric Neurology, Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom,* [5]*Rosie Neonatal Unit, Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom,* [6]*University of Sheffield, Sheffield, United Kingdom.*

Biallelic mutations in the *KIAA1109* gene are reported to cause Alkuraya-Kucinskas syndrome (OMIM #617822) characterised by cerebral parenchymal underdevelopment. To date, only a few clinical reports have been published detailing the phenotype of this rare autosomal recessive disorder. Severe loss of function variants were lethal; surviving patients with missense variants had global developmental delay, early-onset epilepsy and arthrogryposis. We report a Kurdish consanguineous family with fetal brain abnormalities in two pregnancies detected at 20 weeks of gestation. The couple's first female child died at nine months due to complications of respiratory tract infection; she had severe global developmental impairment with hypotonia and infantile epilepsy. Antenatally detected brain defects were confirmed via MRI head imaging at 1 week of age including severe generalised lissencephaly, parenchymal thinning, Dandy-Walker malformation, colpocephaly, and absent corpus callosum. Trio exome sequencing reported compound heterozygosity for a protein-truncating variant (c.3673C>T;p.(Arg1225Ter)), and a variant of uncertain significance (c.2794-21C>G) in the *KIAA1109* gene predicted to affect splicing. The second pregnancy was diagnosed with similar brain defects including mild ventriculomegaly, absent corpus callosum, lack of sulcation and sylvian fissure formation, thick neuronal eminence with lack of migration, small cerebellum, and kinked brainstem. The pregnancy was terminated at 21 weeks of gestation with subsequent testing confirming compound heterozygosity for the familial *KIAA1109* variants. This report expands the mutational spectrum of Alkuraya-Kucinskas syndrome and emphasises its importance as a differential diagnosis of antenatally detected neural migration defects, especially in the presence of a severe cerebral pattern mimicking tubulinopathies (Cabet et. al, 2019).

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P09.009.B Whole-exome sequencing reveals differential enhancement of ion channels activity genes between Alzheimer patients and controls

Dimitar Serbezov[1], *Maja Atanasoska*[2], *Lubomir Balabanski*[1,2], *Sena Karachanak-Yankova*[1,3], *Radoslava Vazharova*[4,2], *Dragomira Nikolova*[1], *Marta Mihaylova*[1], *Rada Staneva*[1], *Olga Antonova*[1], *Vera Damyanova*[1], *Mihail Ganev*[1], *Viktoria Spasova*[1], *Dessislava Nesheva*[1], *Zora Hammoudeh*[1], *Savina Hadjidekova*[1], *Diyana Belezhanska*[1],

Shima Mehrabian[1], Maria Petrova[1], Latchezar Traykov[1], Draga Toncjeva[1]

[1]Medical University of Sofia, Sofia, Bulgaria, [2]Gynecology and assisted reproduction hospital "Malinov", Sofia, Bulgaria, [3]Department of Genetics, Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria, [4]Department of Biology, Medical genetics and Microbiology, Faculty of Medicine, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria.

Introduction: Alzheimer disease (AD) is the most common cause of dementia, and identifying genetic factors causing changes in molecular pathways associated with AD are crucial for developing diagnostic methods and treatment therapies.

Materials & Methods: Whole-exome sequencing was performed on two DNA pools, one set up with DNA isolated from 66 AD patients and the other from 100 individuals showing no symptoms of dementia. Gene enhancement was performed using genes with variants with very low European population frequencies (< 0.0025) but with higher estimated frequency in our pools (> 0.01), Associated with these genes molecular functions and pathways were determined using the online platforms *Toppgene* and *Reactome*.

Results: The molecular function with the largest number of rare variant genes in both pools was determined to be *Ribonucleotide binding*. Neuronal system pathways were subsequently found to be differentially enhanced in the pools. More specifically, *HCN channels*, *Long-term potentiation*, and *Assembly and cell surface presentation of NMDA receptors* are pathways notably enhanced in the control group compared to the AD patients group.

Conclusions Our results reveal differential enhancement of ion activity genes between Alzheimer patients and controls, lending support to the ion channel hypothesis of AD. **Acknowledgment** KP-06-N33/5 from 13.12.2019 - National Science Fund of Bulgaria"

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P09.010.C Identifying serum biomarkers of neurological disorders using whole genome sequencing

Grace Png*[1,2], *Andrei Barysenka[1], Linda Repetto[3], Pau Navarro[4], Xia Shen[5,6,7], Emmanouil Tsafantakis[8], Maria Karaleftheri[9], George Dedoussis[10], Anders Mälarstig[11,12], James F. Wilson[5,13], Arthur Gilly[1], Eleftheria Zeggini[1,2]

[1]Institute of Translational Genomics, Helmholtz Zentrum Munich, Neuherberg, Germany, [2]TUM School of Medicine, Technical University of Munich and Klinikum Rechts der Isar, Munich, Germany, [3]Centre for Global Health Research, Usher Institute, University of Edinburgh, Western General Hospital, Edinburgh, United Kingdom, [4]MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, United Kingdom, [5]Centre for Global Health Research, Usher Institute, University of Edinburgh, Edinburgh, United Kingdom, [6]Biostatistics Group, State Key Laboratory of Biocontrol, School of Life Sciences, Sun Yat-sen University, Guangzhou, China, [7]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden, [8]Anogia Medical Centre, Anogia, Greece, [9]Echinos Medical Centre, Echinos, Greece, [10]Department of Nutrition and Dietetics, School of Health Science and Education, Harokopio University of Athens, Athens, Greece, [11]Department of Medicine, Karolinska Institutet, Solna, Sweden, [12]Emerging Science & Innovation, Pfizer Worldwide Research, Development and Medical, Cambrige, MA, USA, [13]MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine, University of Edinburgh, Western General Hospital, Edinburgh, United Kingdom.

Introduction: Despite the increasing global burden of neurological disorders, there is a lack of effective diagnostic and therapeutic biomarkers. Proteins are often dysregulated in disease and have a strong genetic component; here, we show that the human serum proteome is an accessible reservoir of potential biomarkers.

Materials and Methods: Using deep whole genome sequencing (WGS) data from two population-based cohorts (15X WGS; N = 2,893), we carry out a protein quantitative trait locus (pQTL) analysis of 184 neurologically-relevant proteins. We then apply causal inference tools, such as colocalisation analysis, to map these loci to neurological diseases.

Results: We detect 139 pQTLs for 107 proteins, the majority of which (65%) are *cis*-acting, including 76 independently-associated sequence variants that have not been previously identified. We observe causal associations between serum levels of CD33 and Alzheimer's disease, GPNMB and Parkinson's disease, and MSR1 and schizophrenia, describing their clinical potential and highlighting drug repurposing opportunities.

Conclusions: We have generated a pQTL map of neurological proteins, elucidating the genetic landscape that underlies the circulating proteome and its connection to neurological disorders. In doing so, we confirm known protein-disease associations, and describe new potential mechanisms that contribute to disease aetiology.

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P09.011.D Alzheimer's disease polygenic risk score assessment on longitudinal amyloid load in cognitively intact older adults

Emma Susanne Luckett*[1], *Jolien Schaeverbeke[1], Lars Bertram[2], Koen Van Laere[1,3], Patrick Dupont[1], Isabell Cleynen[1], Rik Vandenberghe[1,3]

[1]KU Leuven, Leuven, Belgium, [2]University of Lübeck, Lübeck, Germany, [3]UZ Leuven, Leuven, Belgium.

Introduction: Published data have highlighted associations between Alzheimer's Disease (AD) susceptibility loci and AD-related brain changes. We investigated these associations within the Flemish Prevent AD Cohort KU Leuven (F-PACK) using AD polygenic risk scores (PRSs) and longitudinal brain amyloid load.

Materials and methods: Sixty-one cognitively healthy participants (age: 68 (56-79), 29 females, 29 *APOE ε4* carriers) received an [^{18}F]Flutemetamol-PET scan at two timepoints (interval 4.6 years (3.4-8.6)). Amyloid rate of change: absolute change divided by interval. Genotyping performed using Illumina GSA and imputation using the Michigan server and HRC reference panel. Data underwent standard quality control. PRSice was used for PRS calculations with Stage 1 summary statistics from Kunkle et al. (2019) as base file and European individuals from 1000 Genomes (N = 503) as reference for clumping. We calculated PRSs at three thresholds for SNP inclusion (pT): pT = 0.5 (Escott-Price et al. 2015); $1x10^{-5}$; $5x10^{-8}$. Linear regression models determined if PRSs were associated with amyloid rate of change at each pT (age, sex and first three PCs as covariates).

Results: There was a significant effect of PRS on amyloid rate of change when using the more stringent thresholds for SNP

inclusion: pT = 5x10^{-8}: β = 0.0054 (CI: 0.000042-0.011), $p = 0.048$; pT = 1x10^{-5}: β = 0.0056 (CI: 0.0000785-0.011), $p = 0.047$. Amyloid accumulation, however, was not significantly associated with PRS when pT = 0.5: β = 0.0013 (-0.004-0.007), $p = 0.619$.

Conclusions: A significant effect of PRS is detected with more stringent pTs, suggesting AD-related brain changes have an oligogenic architecture, in line with recent publications showing AD is an oligogenic rather than polygenic disease.

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P09.012.A Pathway based enrichment analysis of genes associated with Alzheimer's disease

David Vogrinc, *Katja Goričar, Vita Dolžan*

University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia.

Introduction: Alzheimer's disease (AD) is the most common neurodegenerative brain disease affecting millions worldwide. Late-onset AD cases comprise the vast majority of all AD patients. Although family history is important in assessing AD risk, complex genetic and environmental interactions contribute to AD even late in life. Our aim was to perform pathway enrichment analysis on a defined AD risk gene set, obtained from comprehensive literature review.

Materials and Methods: We performed a literature synthesis of genome wide association studies and their meta-analyses that investigated AD susceptibility. Next, we performed a functional enrichment analysis, based on Gene Ontology: Biological Process. Pathway network was visualized using Cytoscape software.

Results: 105 loci were associated with AD risk, while 30 additional loci were associated with biomarker levels in body fluids or neurologic features. Identified genes were grouped into four parental categories for AD risk gene set and seven parental categories for AD biomarker gene set. Common categories for both gene sets were metabolic process, cellular process, localization and biological regulation, while transport, neurological system process and regulation of cellular process were additionally identified in biomarker gene set.

Conclusion: Functional enrichment analysis enabled us to identify key biological processes of AD pathogenesis. Our comprehensive review can serve as a basis for studies of pathophysiological mechanisms of risk genes, identified on a genome-wide scale. Better characterization of risk genes could enable the stratification of patients according to the main molecular mechanisms of pathogenesis, supporting development of tailored and personalised treatment of AD. Research grants: ARRS P1-0170.

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P09.013.B 'Genetically predicted telomere length is associated with age- and Alzheimer's Disease-related brain structure alterations.'

Blanca Rodríguez-Fernández[1], *Natalia Vilor-Tejedor*[1,2,3], *Marina García*[1], *Grégory Operto*[1,4,5], *Eider M. Arenaza-Urquijo*[1,4,5], *Carolina Minguillon*[1,4,5], *Karine Fauria*[1,5], *Immaculata De Vivo*[6,7], *Arcadi Navarro*[1,8,9], *José Luis Molinuevo*[1,10], *Juan D. Gispert*[1,4,11], *Aleix Sala-Vila*[1,4], *Marta Crous-Bou*[1,6,12], *for the ALFA study*

[1]*Barcelonaβeta Brain Research Center - Pasqual Maragall Foundation, Barcelona, Spain,* [2]*Centre for Genomic Regulation (CRG). The Barcelona Institute for Science and Technology, Barcelona, Spain,* [3]*Erasmus University Medical Center, Department of Clinical Genetics, Rotterdam, Netherlands,* [4]*IMIM - Hospital del Mar Medical Research Institute, Barcelona, Spain,* [5]*Centro de Investigación Biomédica en Red de Fragilidad y Envejecimiento Saludable (CIBER-FES), Madrid, Spain,* [6]*Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, USA,* [7]*Channing Division of Network Medicine, Harvard Medical School, Boston, MA, USA,* [8]*Institute of Evolutionary Biology (CSIC-UPF), Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona, Spain,* [9]*Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain,* [10]*Present address: H. Lundbeck A/S, Valby, Denmark,* [11]*Centro de Investigación Biomédica en Red Bioingeniería, Biomateriales y Nanomedicina, Madrid, Spain,* [12]*Catalan Institute of Oncology (ICO)-Bellvitge Biomedical Research Center (IDIBELL), Hospitalet del Llobregat, Spain.*

Introduction: Telomere length (TL) is an objective biomarker of biological aging. Shorter telomeres are associated with accelerated aging, consequently increasing the risk of age-related diseases such as Alzheimer's Disease (AD). We aimed to test the potential causal role of TL in age- and AD-related brain structure alterations through a Mendelian Randomization (MR) analysis.

Methods: We included 1,134 participants from the ALFA (ALzheimer and FAmilies) study. We calculated composite brain signatures reflecting cortical thickness of specific age or AD vulnerable brain regions. A total of 7 genome-wide hits associated with TL were used as instrumental variables. Causal effects of TL were estimated using the MR-inverse-variance weighted method (IVW). Sensitivity analyses using the MR-Egger method were conducted to test for directional pleiotropic effects. All analyses were adjusted by age, sex and years of education. Stratified analyses by *APOE*-ε4 status were performed.

Results: MR analysis revealed significant associations between genetically predicted shorter TL and reduced cortical thickness in age and AD-related brain signatures; however, evidence for directional pleiotropy was observed. Our results suggested an effect modification by *APOE*-ε4 status: significant effects were only observed among *APOE*-ε4 carriers with no evidence for pleiotropy (AD: $\beta_{IVW} = -7.61$, p-value = 0.027; Aging: $\beta_{IVW} = -8.10$, p-value < 0.001).

Conclusions: Our results suggest a causal role of telomeres in vulnerability of brain regions associated with aging processes and AD, specifically in individuals at increased genetic risk of AD.

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P09.014.C Genomic data suggests the involvement of TLR5 variants in modifyingthe risk for Alzheimer's disease

Dragomira N. Nikolova[1], *Dimitar Serbezov*[1], *Mihail Ganev*[1], *Sena Karachanak-Yankova*[1], *Marta Mihaylova*[1], *Shima Mehrabian*[2], *Maria Petrova*[2], *Vera Damyanova*[1], *Diyana Belezhanska*[2], *Lachezar Traykov*[2], *Savina Hadjidekova*[1], *Draga Toncheva*[1]

[1]*Department of Medical genetics, Medical University - Sofia, Sofia, Bulgaria,* [2]*Department of Neurology, University Hospital "Alexandrovska", Medical University - Sofia, Sofia, Bulgaria.*

Introduction: Alzheimer's disease (AD) is an irreversible, progressive brain disorder leading to dementia in adults. Immune system's dysregulation is a key factor in the AD pathogenesis,

particularly Toll-like receptors (TLRs) which participate in neuroinflammatory reactions. The TLR-mediated response has beneficial roles stimulating phagocytosis releasing neurotoxic products. he aim of our study was to determine the presence and significance of variants in TLR genes in the genomic data of patients with AD.

Materials and Methods: We selected 127 genes associated with PRR (pattern recognition receptors) pathways, innate and adaptive immunity, inflammatory responses, defense responses to viruses and bacteria. Whole exome sequencing was performed on two DNA pools, one constructed with DNA from AD patients and the other with DNA from healthy age-synchronized individuals. The obtained genomic data was then surveyed for the presence of PRR variants and their frequency in the two pools was calculated.

Results: 1229 PRR variants were detected in both pools, but only 24 of them were significantly different in frequency between the two pools. Of these 5 variants belonged to different TLR family gene members - rs5744168 and rs2072493 (*TLR5)*; rs179008 (*TLR7)*; rs3764880 (*TLR8)*; rs5743611 (*TLR1)*. Two variants of highest p-value belonged to *TLR5* - rs5744168 (FDR = 0.002) and rs2072493 (FDR = 0.005).

Conclusion. Our study demonstrates the role of TLRs in AD pathogenesis. The role of two *TLR5* variants can be highlighted - rs5744168, a possibly protective factor and rs2072493, a risk factor. **Acknowledgement**: The study is a part of a project KP-06-N33/5 from 13.12.2019 - NSF of Bulgaria.

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P09.015.D Analysis of pathogenic variants in Alzheimer's disease related genes in cases, healthy young controls and centenarians

Sena Karachanak-Yankova[1,2], *Dimitar Serbezov*[1], *Lubomir Balabanski*[1,3], *Marta Mihaylova*[1], *Dragomira Nikolova*[1], *Mihail Ganev*[1], *Desislava Nesheva*[1], *Zora Hammoudeh*[1], *Blaga Rukova*[1], *T Dekova*[2], *Diyana Belezhanska*[4], *Shima Mehrabian*[4], *Mariya Petrova*[4], *Latchezar Traykov*[4], *Savina Hadjidekova*[1], *Draga Toncheva*[1]

[1]*Department of Medical Genetics, Medical Faculty, Medical University-Sofia, Sofia, Bulgaria,* [2]*Department of Genetics, Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria,* [3]*Gynecology and assisted reproduction hospital "Malinov", Sofia, Bulgaria,* [4]*Depatment of Neurology, UH "Alexandrovska", Medical University-Sofia, Sofia, Bulgaria.*

Introduction: Along with vascular and oncological diseases, dementia is one of the most significant health and social problems of the 21st century. The most common type of dementia is Alzheimer's disease (AD), which affects 65-70% of patients over 65. In order to deepen the knowledge of the genetic basis of the disease, in the present study we have analyzed pathogenic variants in AD related genes by testing their presence among patients, healthy young individuals and centenarians.

Materials and methods: We have performed whole exome sequencing of three DNA pools of 66 patients, 61 young healthy individuals (mean age 25 years) and 32 centenarians. The sequencing was performed with high coverage (250 x) and the obtained variants were filtered by stringent criteria.

Results: We have focused on the presence of pathogenic variants in AD-related genes, namely *APOE, PSEN1, PSEN2, MAPT, APP* and *TREM2*. The exomes of AD patients contain 4 pathogenic variants in these genes including the *APOE-ε4* allele. The rs429358 *APOE* allele, which is known to significantly increase AD risk, is also found as one of the two pathogenic variants in healthy young individuals. On the other hand, centenarian exomes contain only one AD pathogenic variant - rs193922916 in *APOE*, which shows recessive inheritance.

Conclusions: The case/control analysis of the presence of pathogenic variants in AD patients and healthy individuals at different age can pave the way towards the implementation of new diagnostic and prognostic blood biomarkers for Alzheimer's disease. Acknowledgment: KP-06-N33/5 from 13.12.2019 - National Science Fund of Bulgaria

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P09.017.B Investigation of proven genetic risk factors in the Hungarian amyotrophic lateral sclerosis population

Zsófia Flóra Nagy[1], *Margit Pál*[1], *Gloria Kafui Esi Zodanu*[1], *Dalma Füstös*[1], *Péter Klivényi*[2], *Márta Széll*[1]

[1]*University of Szeged, Department of Medical Genetics, Szeged, Hungary,* [2]*University of Szeged, Department of Neurology, Szeged, Hungary.*

Introduction: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease which affects both upper and lower motor neurons. ALS has an oligogenic background: several genes and mutations are known to cause ALS, and additionally there are well established genetic risk factors and disease modifying variants for the disease. In this study we investigated 9 independent genetic risk factors of ALS in the Hungarian population.

Patients and methods: 157 patients of Hungarian origin diagnosed with ALS were recruited for this study. For the analysis of the *ATXN1* and *ATXN2* genes repeat sizing was used. MLPA technique was used to screen for the duplications of *SMN1* gene. We reanalyzed whole exome sequencing data from a previous study then used Sanger sequencing to confirm the presence of the detected variants.

Results: We could not confirm the previously detected association between the *CYLD* gene and ALS. We observed a non-significant but tendentious relationship between *ATXN2* intermediate-length repeat expansion and the duplication of the *SMN1* gene and ALS. In the *ANXA11*, *PON1*, *PON3* and *GLT8D1* genes we identified 1-1 relevant variants of uncertain significance (VUS), and in the *TIA1* gene 4 variants were detected; all have been identified as ALS-associated genetic variants. A potentially pathogenic variant in the *MFSD8* gene was revealed.

Discussion: With our results we contribute to the fine mapping of the genetic heterogeneity of ALS. For the development and clinical application of novel therapeutic modalities in ALS it is essential to stratify the patients based on their genetic background. Funding:Hungarian Brain Research Program (Grant No. 2017-1.2.1-NKP-2017-00002).

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P09.018.C journALS: a comprehensive, uniform analysis of three decades of genetics research in amyotrophic lateral sclerosis and frontotemporal dementia

Mark A. Doherty[1], *Ciarán Kelly*[1], *Louisse Mirabueno*[2], *Orla Hardiman*[1], *Russell L. McLaughlin*[1]

[1]Trinity College Dublin, Dublin 2, Ireland, [2]University of Reading, Reading, United Kingdom.

Background: Here we present journALS, a web-application designed to assess the clinical significance of all previously reported amyotrophic lateral sclerosis (ALS)- and frontotemporal dementia (FTD)-associated genetic variants. ALS is a highly heritable neurodegenerative disorder which exhibits a phenotypic spectrum with FTD. Inferring variant significance is complicated in ALS and FTD due to genetic heterogeneity, late age of onset, age-related penetrance and a high proportion of sporadic cases.

Methods: Of 2,914 screened articles, 1,028 were found to be relevant ALS or FTD genetic studies. 3,111 reported variants were identified in 363 genes. Detailed phenotype and variant data and were gathered. 479 pedigrees were documented. Variants in the 363 identified genes were extracted from ALS-specific and general genomics datasets, creating a final database of 1.5 million variants. We uniformly assessed all variants for pathogenicity, penetrance, prevalence, and phenotypic and geographic heterogeneity.

Results: 111 pathogenic or likely pathogenic variants were confirmed in 23 genes, with 10% classified as benign or likely benign; and greater than 89% classified as variants of uncertain significance. 10% of pathogenic or likely pathogenic variants exhibit geographic heterogeneity. We find that due to the high lifetime risk of ALS and low frequency of pathogenic alleles, even the current largest genomics projects will struggle to confidently identify intermediate penetrance rare variants in ALS.

Discussion: As precision treatments targeting specific ALS-causing mutations in specific patients are becoming reality, distinguishing pathogenic and benign ALS and FTD variants becomes essential. Our results support a reorientated view of several ALS genes and variants.

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P09.020.A Impact of emerging diagnostic technologies on diagnostic rate of ataxia: experience of a Hungarian expert centre for rare neurological diseases

Viktor Molnár, *Magdolna Menyei-Kiss, Péter Balicza, Noémi Ágnes Varga, Zoltán Grosz, Barbara Trombitás, Anikó Gál, Mária Judit Molnár*

Semmelweis University, Institute of Genomic Medicine and Rare Disorders, Budapest, Hungary.

Introduction: The diagnostic evaluation of a patient with chronic ataxia is clinically challenging due to its association with a heterogeneous array of neurologic conditions spanning common acquired etiologies to rare genetic disorders. Availability of comprehensive NGS-based testing facilitates the genetic diagnosis, however it is far from perfect choice in covering every single case.

Materials and Methods: Consecutive cases referred with chronic and progressive ataxia from the last 15 years. The dataset with solved and unsolved cases had been enriched with data about clinical phenotype, severity and co-morbidities as well as time course and family history.

Results: In accordance with the literature approximately half of the cases in our dataset with hereditary cerebellar ataxia are caused by spinocerebellar ataxias. The second largest groups are mitochondrial disorders including mtDNA mutations and nuclear mitochondrial gene mutations in FRDA, POLG and SPG7 genes. Some of the familial AR ataxias are found to be related to ATM, SETX, SACS alterations. Further rare causes (such as CTX, APTX, CACNA1A etc.) were detected in single families. Due to its rarity, nonspecific or atypical clinical appearance, some of the successfully revealed AR ataxias were solved by using exome sequencing.

Conclusions: Optimal testing strategy of the ataxia cohort is complicated by (i) highly heterogeneous composition of causes, (ii) population-specific prevalence, and (iii) the need of clinical genetics knowledge. These results provide relevant epidemiological information, bringing a comprehensive knowledge of the most prevalent subtypes of genetic ataxias and their phenotypes in Hungary.

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P09.021.B Genetics of Ataxia Telangiectasia in a Highly Consanguineous Population

Hanouf Omar Aldeeb

KSU, Riyadh, Saudi Arabia.

Ataxia telangiectasia (AT) is a rare autosomal recessive multisystemic disorder. It usually presents in toddler years with progressive ataxia and oculomotor apraxia, or less commonly, in the late-first or early-second decade of life with mixed movement disorders. Biallelic mutations in Ataxia Telangiectasia Mutated gene (*ATM*) cause AT phenotype, a disease not well documented in Saudi Arabia, a highly consanguineous society. We studied several Saudi AT patients, identified *ATM* variants, and investigated associated clinical features. We included 17 patients from 12 consanguineous families. All patients had a comprehensive clinical and radiological assessment, and most were examined through whole-exome sequencing (WES). Selected individuals were analyzed using various genetic approaches. We identified 4 different *ATM* variants in our patients: 3 previously reported mutations, and one novel variant. Nearly all patients had classical AT presentation except for two patients with a milder phenotype. Among the 3 known variants, a deletion causing truncation (c.381delA resulting in p.Val128Ter) was identified in 13 patients. Two patients harboured the other 2 variants, (c.9001_9002delAG resulting in p.Ser3001Phefs*6) and (c.9066delA resulting in p.Glu3023Alafs*10). We speculate that c.381delA is a founder mutation in our population. This study provides a genotype-phenotype relationship in a previously unstudied consanguineous population. Our findings contribute to improve local clinical care, therapy, and genetic counseling. We are grateful to the patients and their families for their participation. This research received intramural funds from KFSHRC, grants from NSTIP/KACST (NK, DC), and KSCDR (NK). We extend our special thanks to the funding agencies.

H.O. Aldeeb: None.

P09.022.C Case report of classic ataxia-telangiectasia with neurological phenotype and rare *ATM* gene variant

Rasa Traberg[1], *Jurate Laurynaitiene*[2], *Darius Cereskevicius*[1], *Rasa Ugenskiene*[1]

[1]*Department of Genetics and Molecular medicine, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania,* [2]*Department of Neurology, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania.*

Background: Classic ataxia-telangiectasia (A-T) is characterized by progressive cerebellar ataxia beginning between ages one and

four years, oculomotor apraxia, choreoathetosis, telangiectasias of the conjunctivae, immunodeficiency, and frequent infections. We present a case were no telangiectasia were evident.

Methods: We report 11-years old boy who was referred to geneticist due to suspected hereditary ataxia. He is the first child of healthy unrelated couple. Perinatal period and early psychomotor development were normal but gait instability was noticeable from 16 month of age and gradually progressed. At 10 year old neurologic examination showed horizontal nystagmus and saccadic eye movements, face hyperkinesia, drooling, choreoathetosis, Romberg instability, gait ataxia. Patient was unable to walk without assistance. Brain MRI revealed cerebellar atrophy, laboratory testing increased alfa fetoprotein - 157 kU/l (normal value 0-7,89). There were no oculocutaneous telangiectasia and immunodeficiency reported. Patient's grandfather was diagnosed with prostate cancer at 50 years old. *ATM* gene sequencing was performed by NGS.

Result: Two compound heterozygous variants in trans position were found: pathogenic variant NM_000051.3(ATM):c.3214G>T;. Glu1072Ter (inherited from father) and variant of unknown significance NM_000051.3(ATM):c.8710G>C ;Glu2904Gln; (inherited from mother). The variant c.8710G>C (p.Glu2904Gln) was not found in the population databases. Alternative variant c.8711A>G (Glu2904Gly), affecting the same amino acid is classified as pathogenic. The variant c.8710G>C was predicted to be deleterious by *in silico* analysis. Based on clinical findings and variant pathogenicity prediction the variant c.8710G>C (p.Glu2904Gln) was classified as likely pathogenic.

Conclusions: The progressive symptoms allowed to suspect and genetic testing confirmed the diagnosis of A-T.

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P09.023.D Novel variants in critical domains of ATP8A2 and expansion of clinical spectrum

Erfan Heidari[1], *Alexander N. Harrison*[2], *Ali Reza Tavasoli*[3], *Navid Almadani*[4], *Robert S. Molday*[2], *Masoud Garshasbi*[1]

[1]*Tarbiat Modares University, Tehran, Iran, Islamic Republic of,* [2]*University of British Columbia, Vancouver, BC, Canada,* [3]*Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of,* [4]*Royan Institute for Reproductive Biomedicine, Tehran, Iran, Islamic Republic of.*

Introduction: ATP8A2 is a member of the P4-ATPase subfamily of P-type ATPases that actively flips phosphatidylserine and phosphatidylethanolamine across membranes to generate and maintain transmembrane phospholipid asymmetry. Loss-of-function variants in *ATP8A2* cause severe neurodegenerative and developmental disorders in rodents and humans.

Results: whole exome sequencing combined with homozygosity mapping on DNA isolated from understudied patients unravel one splicing variant (c.1868-2A>G) and two missense variants (p.Asp825His and p.Met438Val) reside in highly conserved domains of *ATP8A2* gene in three unrelated Iranian families that cause intellectual disability, dystonia, microcephaly/below-average head size, mild optic atrophy, difficult feeding and developmental delay. Furthermore, all the affected individuals displayed tooth abnormalities associated with defects in teeth development. Protein expression and functional studies indicate that p. Asp825His variant leads to a very low expression level of ATP8A2 and lack of phosphatidylserine-activated ATPase activity. Moreover, we confirmed that p.Met438Val and p.Asp825His variants lead to rapid degradation of the misfolded ATP8A2 by proteasomes.

Conclusion: We conclude that Asp825 which coordinates to the Mg^{2+} ion within the ATP binding site and Met438 are essential for the proper folding of ATP8A2 into a functionally active phosphatidylserine flippase. Also, our study for the first time provides evidence on the association of tooth abnormalities with defects in ATP8A2 suggesting that disruption of ATP8A2 flippase activity interferes with teeth development and therefore expanding the clinical spectrum of the associated disease.

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P09.024.A Unravelling the implication of the major vault protein in neuroanatomical phenotypes at the autism associated 16p11.2 locus

Binnaz YALCIN[1], *Perrine Kretz*[2], *Christel Wagner*[2], *Charlotte Montillot*[1], *Sylvain Hugel*[3], *Ilaria Morella*[4], *Meghna Kannan*[2], *Anna Mikhaleva*[5], *Marie-Christine Fischer*[2], *Maxence Milhau*[1], *Riccardo Brambilla*[4], *Yann Herault*[2], *Alexandre Reymond*[5], *Mohammed Selloum*[6], *Stephan Collins*[1]

[1]*Inserm, Dijon, France,* [2]*IGBMC, Illkirch, France,* [3]*INCI, Strasbourg, France,* [4]*NMHRI, Cardiff, United Kingdom,* [5]*Center for Integrative Genomics, Lausanne, Switzerland,* [6]*MCI, Illkirch, France.*

Introduction: Using mouse genetic studies, we set out to identify which of the 30 genes causes brain size and other NeuroAnatomical Phenotypes (NAPs) at the autism-associated 16p11.2 locus, independently in male and female.

Materials and methods: To assess NAPs, we developed or acquired through collaboration single-gene heterozygous knockout mice, representing 20 unique genes of the 16p11.2 locus. For the remaining 10 genes, the germline transmission of the mutation failed despite multiple attempts or no mouse model was available during the course of the study. Result: Here we show that multiple genes mapping to this region regulate brain size in contrast to previous studies, with female significantly less affected. Major Vault Protein (MVP), the main component of the vault organelle, is a highly conserved protein found in higher and lower eukaryotic cells, yet its function is not understood. While we find MVP expression highly specific to the limbic system, *Mvp* stood out as the top driver of NAPs, regulating the morphology of neurons, postnatally and specifically in male. Finally, we demonstrate that the double hemideletion *Mvp*::*Mapk3* rescues NAPs and alters behavioral performances, suggesting that MVP and ERK share the same pathway, in vivo.

Conclusion: Our results highlight that sex-specific neuroanatomical mechanisms must be considered in neurological disorders such as autism and provide the first evidence for the involvement of the vault organelle in the regulation of the mammalian brain size.

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P09.025.B Contribution of compound heterozygous *CACNA1H* mutations in autism spectrum disorder susceptibility

Marta Viggiano[1], *Cinzia Cameli*[1], *Annio Posar*[2,3], *Maria C. Scaduto*[2], *Magalì Rochat*[4], *Paola Visconti*[2], *Elena Bacchelli*[1], *Elena Maestrini*[1]

[1]*Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy,* [2]*IRCCS Istituto delle Scienze Neurologiche di Bologna, UOSI Disturbi dello Spettro Autistico, Bologna, Italy,* [3]*Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy,* [4]*IRCCS Istituto delle Scienze Neurologiche di Bologna, Diagnostica Funzionale Neuroradiologica, Bologna, Italy.*

Introduction: Autism Spectrum Disorder (ASD) is a complex neuropsychiatric disorder with a strong genetic component. So far, more than one hundred high-confidence susceptibility genes have been identified and recent efforts have led to an ever-growing list of ASD candidate genes. Among these, low-voltage activated T-type calcium channels (Ca_v3) genes (*CACNA1G*, *CACNA1H*, *CACNA1I*) have been consistently implicated, which nicely correlates with the role calcium signaling in neuronal function.

Materials and Methods: We performed whole genome sequencing analysis in a cohort of 105 families, consisting of 125 ASD individuals, 210 parents and 57 unaffected siblings, to explore the presence of rare damaging variants in Ca_v3 genes.

Results: We have identified inherited damaging variants in Ca_v3 genes in 21 ASD families. Interestingly, compound heterozygous rare damaging missense variants were detected in the *CACNA1H* gene in 6 ASD subjects (2 sibs, 2 MZ twins and 2 independent cases), belonging to 4 different families. The identified biallelic damaging variants could affect the *CACNA1H* protein activity with a recessive model and contribute to the disease development in the context of a high-risk genetic background. Thus, we are performing functional analysis to clarify the role of the *CACNA1H* variants on the calcium channel activity.

Conclusions: The identification of biallelic mutations in 4 different ASD families provides further support for a role of *CACNA1H* in ASD susceptibility, and for the first time highlights it as a candidate gene in ASD, acting in a recessive mode of inheritance.Supported by the Italian Ministry of Health (Grant GR-2013-02357561).

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P09.027.D Mitochondrial DNA influences the susceptibility to Autism Spectrum Disorders and the severity of the clinical phenotype

Leonardo Caporali[1], *Claudio Fiorini*[1,2], *Flavia Palombo*[1], *Flavia Baccari*[3], *Martina Romagnoli*[1], *Paola Visconti*[4], *Annio Posar*[4], *Maria Cristina Scaduto*[4], *Elena Maestrini*[5], *Cinzia Cameli*[5], *Marta Viggiano*[5], *Anna Olivieri*[6], *Antonio Torroni*[6], *Elena Bacchelli*[5], *Magali Rochat*[7], *Valerio Carelli*[1,2], ***Alessandra Maresca***[1]

[1]*IRCCS Istituto delle Scienze Neurologiche di Bologna, Programma di Neurogenetica, Bologna, Italy,* [2]*Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy,* [3]*IRCCS Istituto delle Scienze Neurologiche di Bologna, UOSI Epidemiologia e Statistica, Bologna, Italy, Bologna, Italy,* [4]*IRCCS Istituto delle Scienze Neurologiche di Bologna, UOSI Disturbi dello Spettro Autistico, Bologna, Italy, Bologna, Italy,* [5]*Department of Pharmacy and Biotechnology, University of Bologna, Italy, Bologna, Italy,* [6]*Department of Biology and Biotechnology "L. Spallanzani", University of Pavia, Pavia, Italy, Bologna, Italy,* [7]*IRCCS Istituto delle Scienze Neurologiche di Bologna, Programma Diagnostica Funzionale Neuroradiologica, Bologna, Italy, Bologna, Italy.*

Introduction: Autism spectrum disorders (ASD) are complex and lifelong heterogeneous neurodevelopmental conditions. Different genetic models could explain ASD, ranging from monogenic disorder or copy number variation to polygenic disease. Mitochondrial DNA (mtDNA) may have a role in the pathogenesis of ASD.

Materials and Methods: Our cohort consists of 98 families including 117 subjects with ASD, 193 parents and 59 unaffected siblings. We performed deep sequencing of mtDNA, defining haplogroups and evaluating private variants, including those at low heteroplasmy. An independent cohort of 127 Italian families was used as a replica. Both these cohorts were compared to a control group of 5088 healthy individuals. MtDNA content was assessed in blood cells. Multivariable regression was used to evaluate risk factors influencing ASD severity classified by the calibrated severity score of Autism Diagnostic Observation Schedule.

Results: Haplogroup H in probands resulted protective for ASD, counterbalanced by increased risk conferred by haplogroups L and I. Paternal haplogroups U5a and K increased the risk of developing ASD in offspring. Probands showed increased number of missense mutations in MT-ATP6 and MT-ATP8 and reduced mtDNA content. Paternal super-haplogroups H and JT are associated with mild phenotypes, whereas variants with 15%-5% heteroplasmy are associated with severe phenotypes.

Conclusions: Our results indicate a contribution of mtDNA to ASD susceptibility and phenotypic expression. Paternal mtDNA influences the ASD pathogenesis, possibly due to accumulation of nuclear de novo variants or epigenetic alterations in fathers' germinal cells that are transmitted to the offspring. Supported by the Italian Ministry of Health (GR-2013-02357561).

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P09.028.A Classification and exome sequencing of developmental brain Disorders (DBD): novel genes, phenotype expansions and remarkable genetic heterogeneity

Ghaydda Mirzaa[1], *Kimberly Aldinger*[2], *Andrew Timms*[2]

[1]*University of Washington, Seattle, WA, USA,* [2]*Seattle Children's Research Institute, Seattle, WA, USA.*

Developmental brain disorders (DBD) are collectively common pediatric disorders with a high rate of morbidity and mortality among affected individuals. These disorders include brain growth abnormalities such as microcephaly (MIC), megalencephaly (MEG), and malformations of cortical development (MCD; such as lissencephaly, polymicrogyria). The diagnosis of these disorders is often challenging due to the complexity of the clinical and neuroimaging features of these malformations, the rarity of the individual disorders or phenotypes, as well as the rapidly expanding genetic landscape; the identification of which is primarily due to the increasing use of Next Generation Sequencing (NGS) methods. We performed exome sequencing (ES) on >500 families with developmental brain disorders (DBD) between the years of 2013-2020. We analyzed ES data using a custom in-house research pipeline searching for recessive (homozygous, compound heterozygous), dominant/*de novo*, and X-linked variants. We developed a hierarchical clinical classification scheme based on the underlying biological mechanisms including cellular pathways, as well as known associations of features or malformations. We identified several novel genes for DBD broadly, in

addition to numerous candidate genes for MEG, MIC, MCD and other DBD. We also identified many "atypical presentations" or "phenotype expansions" of known syndromic forms of brain malformations. Our series substantially expands on the molecular and phenotypic spectrum of DBD and highlights the combinatorial strength of accurate evidence-based curation of genetic variation and accurate phenotyping of brain malformations by neuroimaging. Our data will highlight important insights that will aid in the future clinical and molecular diagnosis of affected families.

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P09.029.B RNA sequencing profiling in prefrontal brain cortex ofC9ALS/FTD

Maria Isabel Alvarez[1], *Francisco Garcia-Garcia*[2], *Rubén Grillo-Risco*[2], *Marta Gracia*[1], *Laia Rodriguez-Revenga*[1]

[1]*Hospital Clinic of Barcelona, Barcelona, Spain,* [2]*Centro de investigación Principe Felipe, Valencia, Spain.*

Introduction: The GGGGCC (G4C2) repeat expansion in the non-coding region of the *C9orf72* gene is the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) (C9ALS/FTD). Although the mechanisms of disease of C9ALS/FTD remain unknown, a gain of function of a toxic mRNA and RAN-translation have been proposed as triggering pathological mechanisms.

Material and Methods: To further elucidate the mechanisms underlying C9ALS/FTD we performed an RNA sequencing study in prefrontral brain cortex samples from 20 C9ALS/FTD and 12 individuals without neurological manifestation and normal *C9orf72* repeat alleles.

Results: Preliminary data analysis showed fifty one genes differentially expressed between both groups (FDR < 0.05). Among them, 46 were protein coding genes and 5 were non-protein coding. Functional profiling showed deregulated GO annotations in C9ALS/FTD patients including 11 genes involved in *Generation of neurons* and *Neurogenesis* biological processes and 5 genes involved in *Postsynapse* cellular component GO annotations.

Conclusions: Our findings provide additional evidence of genes deregulated in C9ALS/FTD patients that might shed light on neuropathological mechanisms underlying *C9orf72* expansion. Acknowledgements: We thank brain donors and relatives for generous donation and the Neurological Tissue Bank of the Biobanc-Hospital Clinic-IDIBAPS. This work was supported by the Instituto de Salud Carlos III (PI17/01067), co-financed by Fondo Europeo de Desarrollo Regional (FEDER) "una manera de hacer Europa" and AGAUR (2017 SGR1134). The CIBERER is an initiative of the Instituto de Salud Carlos III

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P09.030.C The complexities of *CACNA1A* in clinical neurogenetics

Marina P. Hommersom[1,2], *T. H. van Prooije*[3,2], *M. Pennings*[1], *M. I. Schouten*[1], *H. van Bokhoven*[1,2], *E-J. Kamsteeg*[1], *B. P. C. van de Warrenburg*[3,2]

[1]*Department of Human Genetics, Radboud university medical center, Nijmegen, Netherlands,* [2]*Donders Institute for Brain, Cognition and Behaviour, Nijmegen, Netherlands,* [3]*Department of Neurology, Radboud university medical center, Nijmegen, Netherlands.*

Variants in *CACNA1A* are classically related to episodic ataxia type 2, familial hemiplegic migraine type 1 or spinocerebellar ataxia type 6. Over the years, *CACNA1A* has been associated with a broader spectrum of phenotypes including epilepsy, intellectual disability, and neurological episodic syndromes during childhood. Targeted analysis and unbiased sequencing of *CACNA1A* result not only in clear molecular diagnoses, but also in large numbers of variants of uncertain significance, or likely pathogenic variants where the phenotype does not directly match the *CACNA1A* spectrum. Over the last years, targeted and clinical exome sequencing in our center has identified 41 *CACNA1A* variants. Variant interpretation was based on the ACMG guidelines. Types of *CACNA1A* variants were exon deletions (n = 3), frameshift (n = 6), missense (n = 22), nonsense (n = 6), and splice site (n = 4) variants. Ultimately, variants were considered pathogenic or likely pathogenic in 23 cases, with most phenotypes ranging from episodic or progressive ataxia to more complex ataxia syndromes, as well as with phenotypes dominated by non-cerebellar features such as intellectual disability and epilepsy. In two cases, the causality of the variant was discarded based on non-segregation or an alternative diagnosis. In the remaining 16 cases, the variant was classified as uncertain, due to lack of segregation analysis or uncertain association with a non-classical phenotype. *CACNA1A* thus represents a complex gene in clinical neurogenetics. Accessible functional read-outs are clearly needed, especially in cases with a non-classical phenotype. This work was supported by a grant from Radboud university medical center and Donders Institute for Brain, Cognition, and Behaviour.

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P09.031.D A case of *CACNA1B* associated neurodevelopmental disorder with seizure and non-epileptic hyperkinetic movements

Mehmet Burak Mutlu[1], *Murat Erdogan*[2], *Aslihan Kiraz*[2], *Mohamedahmed Salih Hassan*[1], *Arslan Bayram*[3], *Fatma Demiryılmaz*[1]

[1]*Detagen Genetic Diseases Diagnosis Center, Kayseri, Turkey,* [2]*Medical Genetic Department, Kayseri City Hospital, Kayseri, Turkey,* [3]*Medical Genetic Department, Etlik Zübeyde Hanım Women's Health Teaching and Research Hospital, Ankara, Turkey.*

Introduction: *CACNA1B* associated Neurodevelopmental Disorder with Seizures and Non-Epileptic Hyperkinetic Movements (NEDSNEH, OMIM #618497) is a very rare type of developmental and epileptic encephalopathy with autosomal recessive inheritance. It is a severe neurological disorder characterized by psychomotor developmental retardation, early-onset refractory seizures and non-epileptic hyperkinetic movement disorders, including myoclonus dystonia and dyskinesia, usually manifested by inability to walk and speak and caused by biallelic variants of the *CACNA1B* (Calcium Channel, Voltage-dependent, N-Type, Alpha-1b Subunit) gene.

Materials and Methods: Two siblings with similar findings who were being followed up in the pediatric neurology clinic due to loss of speech, cognitive and motor development retardation were consulted for genetic evaluation. Result: Karyotype analysis, *FMR1* gene analysis and microarray analysis of the two siblings were performed, no abnormality was found. Homozygous pathogenic c.5811dupT, p.Val1938fs (ENST371355) variant detected in the *CACNA1B* gene in the whole exome sequencing analysis.

Conclusions: The variant found in our patients was c.5811dupT; p.Val1938fs is the novel variant previously not reported in the

literature. Up to now, 5 pathogenic variants were reported to be cause of *CACNA1B* associated NEDSNEH, and we present this novel variant detected in our case as the 6th variant. Due to the scarce number of reports describing *CACNA1B* associated NEDSNEH, this variant and the clinical findings of the patients described in this work will contribute to the expansion of the genetic and clinical spectrum of the disease.

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P09.032.A Genetic analysis of Portuguese patients with Cerebellar Ataxia, Neuropathy, Vestibular Areflexia Syndrome (CANVAS)

Ana Lopes[1], *Maria João Malaquias*[2], *Luís Braz*[3,4], *Joana Damásio*[1,2], *Daniela Garcez*[5], *Bravo Marques*[5,6], *André Caetano*[7], *Ana Paula Sousa*[8], *Ana Aires*[3,4], *Ana Graça Velon*[9], *Ana Luísa Sousa*[10], *Cristina Alves*[2], *Cristina Costa*[11], *Cristina Rosado Coelho*[12], *Eva Brandão*[10], *Goreti Nadais*[3], *Joana Guimarães*[3,4], *Michel Mendes*[9], *Nuno Vila-Chã*[2], *Pedro Abreu*[3,4], *Pedro Castro*[3,4], *Raquel Barbosa*[7], *Ricardo Taipa*[13], *Rui Araújo*[3,4], *Teresa Pimentel*[12], *Jorge Pinto Basto*[14], *Sara Morais*[1], *João Parente Freixo*[1], *Marina Magalhães*[2,15], *Jorge Oliveira*[1]

[1]*Centro de Genética Preditiva e Preventiva (CGPP), Instituto de Biologia Celular e Molecular (IBMC), Instituto de Investigação e Inovação em Saúde (i3S), Universidade do Porto, Porto, Portugal, Porto, Portugal,* [2]*Serviço de Neurologia, Centro Hospitalar Universitário do Porto, Porto, Portugal, Porto, Portugal,* [3]*Serviço de Neurologia, Centro Hospitalar Universitário de São João, Porto, Portugal, Porto, Portugal,* [4]*Departamento Neurociências Clínicas e Saúde Mental da Faculdade de Medicina da Universidade do Porto, Porto, Portugal, Porto, Portugal,* [5]*Serviço de Neurologia, Instituto Português de Oncologia de Lisboa Francisco Gentil, Lisboa, Lisboa, Portugal,* [6]*Unidade de Neuropsiquiatria, Fundação Champalimaud, Lisboa, Portugal, Lisboa, Portugal,* [7]*Serviço de Neurologia, Centro Hospitalar de Lisboa Ocidental, Lisboa, Portugal, Lisboa, Portugal,* [8]*Serviço de Neurofisiologia, Centro Hospitalar Universitário do Porto, Porto, Portugal, Porto, Portugal,* [9]*Serviço de Neurologia, Centro Hospitalar de Trás-os-Montes e Alto Douro, Vila Real, Portugal, Vila Real, Portugal,* [10]*Serviço de Neurologia do Centro Hospitalar Entre Douro e Vouga, Santa Maria da Feira, Portugal, Santa Maria da Feira, Portugal,* [11]*Serviço de Neurologia, Hospital Prof. Doutor Fernando da Fonseca, Lisboa, Portugal, Lisboa, Portugal,* [12]*Serviço de Neurologia, Centro Hospitalar de Setúbal, Setúbal, Portugal, Setúbal, Portugal,* [13]*Unidade de Neuropatologia, Centro Hospitalar Universitário do Porto, Porto, Portugal, Porto, Portugal,* [14]*CGC Genetics, Porto, Portugal, Portugal, Porto, Portugal,* [15]*Serviço de Neurologia, Hospital Padre Américo, Centro Hospitalar do Tâmega e Sousa, Penafiel, Porto, Portugal, Penafiel, Portugal.*

Diallelic expansion of an intronic (AAGGG)n in *RFC1* (400_2,000 repeats) has been stablished as a genetic cause of the cerebellar ataxia, neuropathy, vestibular-areflexia syndrome (CANVAS). Four main allelic variants were described: the (AAAAG)11 reference allele (freq.=0.755); expanded AAAAG or AAAGG repeats (freq.=0.130 and 0.079); and the pathogenic (AAGGG)n expansion (freq.=0.007). This relatively high frequency suggests that CANVAS may represent a considerable fraction of late-onset ataxias. Genetic analysis was based on the approach proposed by Cortese *et al.* (2019): (1) a fluorescently labelled PCR was used to amplify the repeat's region; (2) three specific repeat-primed PCRs (RP-PCRs) were performed, each targeting one of the known pentanucleotides - presence of the continuous stutter peak profile in the AAGGG-specific RP-PCR, and absence of similar results in the other two PCRs, is compatible with the diagnosis of CANVAS. From a cohort of 60 clinically suggestive cases of CANVAS (the vast majority previously tested for other ataxias), 35 patients (28 families) were found with a diallelic (AAGGG) n expansion. Five additional cases were carriers for only one expanded pathogenic allele. Mean age-of-onset was 59 ± 10y (range:26-73y). The most common presentation was gait imbalance (n = 33) or sensory symptoms (n = 16). This report describes the first CANVAS patients genetically characterized in Portugal. Our expectation is that this cohort will significantly expand in the short term, as awareness for this clinical entity increases. Testing for the (AAGGG)n expansion in genetically undiagnosed patients with late-onset ataxia (particularly those with the typical clinical triad) is highly recommended.

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P09.033.B CANVAS: the biallelic *RFC1* pentanucleotide repeat expansion in Greek late-onset ataxia patients

Zoi Kontogeorgiou[1], *Chrisoula Kartanou*[1], *Chrisanthi Tsirligkani*[1], *Evangelos Anagnostou*[2], *Michail Rentzos*[3], *Georgia Karadima*[1], *Georgios Koutsis*[1]

[1]*Neurogenetics Unit, 1st Department of Neurology, Eginitio University Hospital, National and Kapodistrian University of Athens, Athens, Greece,* [2]*Clinical Neurophysiology Unit, 1st Department of Neurology, Eginitio University Hospital, National and Kapodistrian University of Athens, Athens, Greece,* [3]*1st Department of Neurology, Eginitio University Hospital, National and Kapodistrian University of Athens, Athens, Greece.*

Cerebellar ataxia, neuropathy and vestibular areflexia syndrome (CANVAS) has been recently linked to a biallelic expansion of a polymorphic pentanucleotide repeat in intron 2 of the replication factor C subunit 1 (*RFC1*) gene. To date, the only clearly pathogenic CANVAS-associated allele includes an $AAGGG_{exp}$ of at least 400 repeats. We presently aimed to detect the aforementioned pathogenic expansion in Greek patients with late-onset ataxia. For this purpose, 77 selected index patients, with late-onset ataxia (age of onset >35 years) and a compatible pedigree, were screened for the expansion. These patients originated from undiagnosed ataxia (67) and neuropathy (10) cohorts referred to the Neurogenetics Unit, 1st Department of Neurology, National and Kapodistrian University of Athens, Greece. Genotyping was performed through fragment and RP-PCR analysis. We report that 5 out of the 77 patients (6.5%) were found homozygous for the pentanucleotide pathological expansion. Moreover we identified two affected siblings raising the total number of genetically confirmed CANVAS cases to 7. Our results confirmed that the AAGGG biallelic expansion is very common in patients with complete CANVAS (80%), but less common in the group with incomplete CANVAS (26.7%), consistent with previous studies. All positive cases exhibited sensory neuropathy as the earliest clinical feature. These results highlight for the first time the presence of the *RFC1* biallelic expansion in Greek patients with late-onset ataxia.

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P09.034.C Novel homozygous *CEP41* mutation in a patient with Joubert syndrome

Ezgi Gökpınar İli[1], *Gonca Bektaş*[2]

[1]*Genetic Diseases Center, Başakşehir Pine and Sakura City Hospital, İstanbul, Turkey,* [2]*Department of Pediatric Neurology, Bakırköy Dr. Sadi Konuk Training and Research Hospital, İstanbul, Turkey.*

Introduction: Joubert syndrome is a genetically heterogenous disorder characterized by hypoplasia of cerebellar vermis with distinctive 'molar tooth sign', hypotonia, developmental delay and neonatal breathing abnormalities.Clinical Report: Two-year-old male was referred to our clinic for global developmental delay. After vaginal delivery at 37th gestational week, he was transferred to newborn intensive care unit for respiratory distress. He obtained head control at 7 months. At two years of age, he was unable to walk, just started to sit without support for small periods and couldn't speak. His parents were consanguineous. Brain MRI revealed "molar tooth sign" of the midbrain in the axial section.

Methods: After written informed consent from the parents, whole exome sequencing (WES) was performed using peripheral blood DNA from the proband.

Results: Because of parental consanguinity, we focused on rare homozygous variants in known Joubert syndrome genes. Homozygous c.911dupC (Glu305ArgfsTer21) mutation in the *CEP41* gene was found. The variant was not found in gnomAD. Since digenic inheritance was suggested for heterozygous mutations in *CEP41* and other ciliopathy-related genes such as *KIF7* and *CC2D2A*, a secondary analysis was performed. An additional heterozygous c.74delA mutation was detected in *KIAA0586* gene which is required for ciliogenesis. Segregation analysis is ongoing.

Conclusions: We report a novel homozygous mutation in *CEP41* and a heterozygous mutation in *KIAA0586* in a patient with Joubert syndrome. It may be beneficial to look for additional mutations for recurrence risk assessment and family planning especially when digenic inheritance is suggested.

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P09.035.D Modelling SCA11 in cells using CRISPR/Cas9: functional validation of a new *TTBK2* missense variant

Daniela Felício[1], *Jorge Sequeiros*[1,2], *Mariana Santos*[1]

[1]*UnIGENe, IBMC - Institute for Molecular and Cell Biology, i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal,* [2]*CGPP, IBMC - Institute for Molecular and Cell Biology, i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal.*

Spinocerebellar ataxia type 11 (SCA11) is a rare autosomal dominant form of cerebellar ataxia, characterized by an almost pure, progressive cerebellar ataxia, abnormal eye movements and impairment of speech. It has been linked to variants in the *TTBK2* gene; all reported cases bear heterozygous truncating variants. *TTBK2* encodes the tau tubulin kinase 2 protein, a protein kinase involved in different cellular processes, e.g., ciliogenesis, microtubule dynamics, and tau and TDP-43 phosphorylation. Currently, the disease mechanism behind SCA11 and *TTBK2* remains unclear. Our group has previously identified a novel missense variant in *TTBK2* (c.625C>T; p.L209F) in two Portuguese siblings with a diagnosis of cerebellar ataxia. Therefore, we aim to characterize the potential pathogenic effect of this variant in SCA11. For that, we created cellular models expressing the endogenous *TTBK2* missense variant, using CRISPR/Cas9. We also inserted a 3xFLAG tag at the N-terminus of TTBK2. Our preliminary results showed that the new variant does not affect the mRNA expression, but causes instead a decrease in TTBK2 protein levels, denoting impairment of protein stability. Thereby, our cellular models may present reduced TTBK2 kinase activity. Indeed, our previous overexpression studies showed that this variant has reduced kinase activity against TDP-43. In conclusion, we created relevant cellular models for study of the molecular and cellular mechanisms underlying SCA11 and, for the first time, linked a missense variant to SCA11. We also believe that abnormal protein phosphorylation may play a key role in SCA11 pathogenesis.

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P09.036.A A systematic review of cerebral phenotypes associated with monogenic cerebral small vessel disease

Ed Whittaker, *Sophie Thrippleton, Liza Y. W. Chong, Victoria Collins, Emily Lancastle, David Henshall, Blair Wilson, Tim Wilkinson, Kirsty Wilson, Cathie Sudlow, Joanna Wardlaw, Kristiina Rannikmäe*

University of Edinburgh, Edinburgh, United Kingdom.

Introduction: Cerebral small vessel disease, an important contributor to stroke and dementia, results from environmental and genetic factors. An emerging minority of cases have a monogenic cause. While *NOTCH3* is the best-known gene, several others have been reported, with less data about their associated phenotypes.

Methods: We performed a systematic review, searching Medline/Embase for any language publications describing *HTRA1*, *TREX1*, *ADA2*, *CTSA* or *COL4A1/2* pathogenic variant carriers. We extracted data about individuals' characteristics, clinical and radiological vascular cerebral phenotypes. We summarised phenotype frequencies per gene, comparing patterns across genes.

Results: We screened 6485 publications and included: 61 *HTRA1* (126 individuals), 35 *TREX1* (123 individuals), 100 *ADA2* (346 individuals), and 5 *CTSA* (14 individuals). Mean ages ranged from 15 (*ADA2*) to 59 years (*HTRA1* heterozygotes). Clinical phenotype frequencies varied widely: stroke 9% (*TREX1*) to 60% (*HTRA1* heterozygotes), cognitive decline 0% (*ADA2*:0/83 adults) to 64% (*CTSA*:9/14 adults), psychiatric features 0% (*ADA2*:0/83 adults) to 100% (*HTRA1* homozygotes/compound heterozygotes:44/44 adults; *CTSA*:14/14 adults). Among individuals with neuroimaging, vascular radiological phenotypes appeared common, ranging from 65% (*ADA2*) to 100% (*HTRA1* homozygotes/compound heterozygotes; *CTSA*) (Table). White matter lesions were the most common pathology. *COL4A1/2* work is ongoing.

Conclusions: There appear to be differences in cerebral manifestations across genes, but this may be due to age and other biases inherent to case reports. The majority of individuals have vascular changes on neuroimaging. **Grants:** KR:MR/S004130/1

*HomZ = homozygous/compound heterozygous; HetZ = heterozygous; N = number; WML = white matter lesions; ICH = intracerebral haemorrhage; PVS = perivascular spaces. *Only those with neuroimaging*

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P09.037.B Deleterious variants in the autophagy regulator *CLEC16A* are associated with microcephaly, brain atrophy, growth retardation and a severe neurodevelopmental disorder

Daphne J. Smits[1], *Rachel Schot*[1], *Martina Wilke*[1], *Marjon van Slegtenhorst*[1], *Brahim Tabarki*[2], *Amal Alhashem*[2], *Antonio Romito*[3], *Aida M. Bertoli-Avella*[3], *Grazia M. S. Mancini*[1]

[1]*Erasmus University Medical Centre, Rotterdam, Netherlands,* [2]*Prince Sultan Military Medical City, Riyadh, Saudi Arabia,* [3]*CENTOGENE GmbH, Rostock, Germany.*

Introduction: The *CLEC16A* (c-type lectin protein 16A) gene locus has frequently been associated with susceptibility to various autoimmune disorders including multiple sclerosis, type - 1 diabetes, rheumatoid arthritis and primary biliary sclerosis. C-type lectin (CLEC) proteins are transmembrane proteins that recognize antigens via their carbohydrate recognition domain and guide them to antigen presenting cells. Unlike other CLEC proteins, CLEC16A lacks an active/full length carbohydrate recognition domain and is instead involved in autophagy and mitophagy. Even though the general link between autophagy and autoimmune diseases is well studied, CLEC16A's physiological function and its role in human disease is still poorly understood.

Methods: With the use of trio whole exome sequencing we identified the first individuals from two unrelated families with bi-allelic loss of function variants in *CLEC16A*.

Results: The affected individuals present with a severe neurodevelopmental disorder including congenital microcephaly, brain atrophy, corpus callosum hypoplasia, growth retardation, hypotonia and a severe developmental delay. In addition, one infant showed severe respiratory infections and died after recurrent and unexplained sepsis.

Conclusion: Our observations suggest a causal implication for the *CLEC16A* variants in these children and confirm the importance of autophagy regulation during human brain development. Descriptions of additional patients with bi-allelic *CLEC16A* variants will definitively associate the gene to this novel neurodevelopmental disorder. We therefore propose to add this gene to exome/genome sequencing panels for microcephaly and severe neurodevelopmental disorders.

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P09.039.D Functional analysis of mutations in a glycosylation enzyme gene, *GFPT1*, underlying limb-girdle congenital myasthenic syndromes (CMS)

Paniz Farshadyeganeh[1], *Bisei Ohkawara*[1], *Masayoshi Kamon*[2], *Toshiyuki Araki*[2], *Hirofumi Komaki*[2], *Kinji Ohno*[1]

[1]*Department of Neurogenetics, School of Medicine, Nagoya University, Nagoya, Japan,* [2]*National Institute of Neuroscience (NCNP), Tokyo, Japan.*

Congenital myasthenic syndromes (CMS) are heterogeneous inherited disorders caused by defective signal transduction at the neuromuscular junction (NMJ). Mutations in more than 30 genes expressed at NMJ have been identified to be involved in CMS. One of CMS, limb-girdle CMS, is caused by mutations in *GFPT1*, a gene encoding glutamine fructose-6-phosphate transferase 1. GFPT1 is a rate-limiting enzyme of the hexosamine biosynthesis pathway to synthesize UDP-N-acetylglucosamine, which is a crucial substrate for glycosylation of proteins and lipids. Post-translational modifications are predicted to be critical for several main components at the NMJ. However, the underlying mechanisms leading to limb-girdle CMS due to GFPT1 deficiency remain elusive. Here, we are dissecting molecular mechanism of 6 missense GFPT1 mutations identified in CMS patients and further investigating one of the missense mutations using patient-derived iPS cells and a knock-in mouse model.

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P09.040.A Chromosomal aberrations in paediatric patients with epilepsy, with or without additional neurodevelopmental disorders: a single-centre clinical investigation

Marlena Młynek[1], *Katarzyna Urbańska*[1], *Katarzyna Pachota*[1], *Petr Berko*[1], *Danuta Sielska-Rotblum*[1], *Dorota Wicher*[1], *Agata Cieślikowska*[1], *Agnieszka Madej-Pilarczyk*[1], *Piotr Iwanowski*[1], *Maria Jędrzejowska*[1,2], *Justyna Pietrasik*[1], *Katarzyna Iwanicka-Pronicka*[1,3], *Anna Gutkowska*[1], *Małgorzata Krajewska-Walasek*[1], *Krystyna Chrzanowska*[1]

[1]*Children's Memorial Health Institute, Department of Medical Genetics, Warsaw, Poland,* [2]*Polish Academy of Sciences, Mossakowski Medical Research Centre, Rare Diseases Research Platform, Warsaw, Poland,* [3]*Children's Memorial Health Institute, Department of Audiology and Phoniatrics, Warsaw, Poland.*

Introduction: Epilepsy is one of the most common neurological disorders affecting up to 1% of the population. A number of genes have been associated with rare autosomal dominant and severe sporadic forms of epilepsy; however, the underlying cause of epilepsy remains unknown in the majority of cases. Copy number variants (CNV) are increasingly recognised as an important aetiology of many human neurodevelopmental disorders, including epilepsy.

Materials and Methods: A whole-genome oligonucleotide microarray (Agilent Technologies 60K and 180K) was applied to a cohort of 239 unrelated patients phenotypically characterised with various type of epilepsy with or without other neurodevelopmental disorders such as developmental delay, intellectual disability, autism, or others.

Results: The chromosomal microarray analysis revealed CNVs considered pathogenic in 43 (18.0%) of affected individuals, ranging from 3.7 kb to 16.9 Mb in size. Of these, 7/43 (16.3%) patients had CNVs in the epilepsy/neurodevelopmental disorder "hotspots" (15q13.3, 15q11–q13, 16p11.2, and 16p13.11), and 4/43 (9.3%) patients have at least two potentially causative CNVs. We identified novel CNVs in genes previously implicated in other neurodevelopmental disorders (*L1CAM*) as well as epileptic encephalopathy (*DENND5A*). In addition, we identified CNVs of uncertain clinical significance in 18/239 (7.5%) of cases.

Conclusions: Our findings correspond with the data reported worldwide and highlight the importance of the whole-genome microarray testing in paediatric population with epilepsy with or without other neurodevelopmental features. Our study confirmed the importance of CNV analysis for the detection of new candidate disease-related genetic regions.

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P09.041.B CSF1R-related adult-onset leukoencephalopathy as an important differential diagnostic factor to consider in early-onset dementias

Dóra Csabán[1], *Péter Balicza*[1], *Anett Illés*[2], *Barbara Trombitás*[1], *Fruzsina Szabó*[1], *Zoltán Grosz*[1], *Mária Judit Molnár*[1]

Frequency of clinical and vascular radiological cerebral phenotypes

	Any clinical cerebral features (%)	Any vascular changes* (%)	WML* (%)	Ischaemia* (%)	ICH* (%)	Enlarged PVS* (%)	Microbleeds* (%)	Atrophy* (%)	Calcification* (%)
HTRA1 HomZ N = 44	80 (35/44)	100 (44/44)	98 (43/44)	34 (15/44)	2 (1/44)	0 (0/44)	41 (18/44)	20 (9/44)	0 (0/44)
HTRA1 HetZ N = 82	76 (62/82)	99 (69/70)	96 (67/70)	66 (46/70)	9 (6/70)	16 (11/70)	27 (19/70)	11 (8/70)	0 (0/70)
TREX1 N = 123	≥54 (≥66/123)	78 (57/73)	89 (65/73)	8 (6/73)	0 (0/73)	0 (0/73)	1 (1/73)	1 (1/73)	32 (23/73)
ADA2 N = 346	42 (144/346)	66 (78/119)	3 (3/119)	44 (52/119)	12 (14/119)	0 (0/119)	0 (0/119)	6 (7/119)	0 (0/119)
CTSA N = 14	100 (14/14)	100 (14/14)	100 (14/14)	57 (8/14)	7 (1/14)	64 (9/14)	21 (3/14)	71 (10/14)	0 (0/14)

[1]*Semmelweis University, Budapest, Hungary,* [2]*PentaCore Laboratory, Budapest, Hungary.*

Introduction: The role of colony-stimulating factor-1 receptor (*CSF1R*) gene is well-known in the background of adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP). ALSP is an autosomal dominant neurodegenerative disorder characterized by dementia, psychiatric symptoms, parkinsonism and behavioral changes. The pathology and symptoms of ALSP overlap with other dementias e.g. frontotemporal dementia (FTD), Alzheimer's disease which may lead to misdiagnosis.

Materials and Methods: 60 patients diagnosed with early-onset dementia (EOD) were tested by next generation sequencing targeted panel, which contained 127 genes associated to neurodegenerative disorders. All patients were examined by experienced board certified neurologists and had brain MRI performed. Patients were clinically diagnosed with AD and FTD.

Results: We detected two rare, potentially pathogenic variants in the *CSF1R* gene. In a male patient, we identified a rare damaging variant [(NM_005211.3):c.2646_2654+6del]. The proband's symptoms were progressive dysphagia, memory impairment, apraxia and spasticity. The other rare variant [(NM_005211.3):c.1771G>A (p.Gly591Arg)] was detected in a female patient. She featured difficulty finding words, cognitive decline and depression. Both detected variants were classified as pathogenic or likely pathogenic according to ACMG.

Conclusion: In our Hungarian EOD cohort two rare damaging variants were identified in the CSF1R gene. Our findings highlight that CSF1R-related ALSP should be included in the differential diagnosis of early-onset dementias. Using comprehensive genetic testing, which simultaneously examine genes associated with different types of dementia could be a feasible and cost effective way to include in the diagnostic workup. This study was supported by KTIA_13_NAP-A-III/6; KTIA_NAP and with the FIKP program.

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P09.043.D Whole-exome sequencing indicates enrichment in MAP kinase activation pathway genes in Bulgarian dementia patients

Marta Mihaylova[1], *Dimitar Serbezov*[1], *Lubomir Balabanski*[2], *Sena Karachanak-Yankova*[3], *Dragomira Nikolova*[1], *Mihail Ganev*[1], *Vera Damyanova*[1], *Desislava Nesheva*[1], *Zora Hammoudeh*[1], *Blaga Rukova*[1], *Diyana Belezhanska*[4], *Shima Mehrabian*[4], *Maria Petrova*[4], *Latchezar Traykov*[4], *Savina Hadjidekova*[1], *Draga Toncheva*[1]

[1]*Department of Medical Genetics, Medical Faculty, Medical University of Sofia, Bulgaria, 2 "Zdrave" s, Sofia, Bulgaria,* [2]*Department of Medical Genetics, Medical Faculty, Medical University of Sofia, Bulgaria, 2 "Zdrave" s, Gynecology and assisted reproduction hospital "Malinov", Sofia, Bulgaria, Sofia, Bulgaria,* [3]*Department of Medical Genetics, Medical Faculty, Medical University of Sofia, Bulgaria, 2 "Zdrave" s, Department of Genetics, Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia, Bulgar-ia, Sofia, Bulgaria,* [4]*Depatment of Neurology, UH "Alexandrovska", Medical University-Sofia, Sofia, Bulgaria., Sofia, Bulgaria.*

Introduction: The aim of this study was to evaluate new genetic and immunological biomarkers for unspecified dementia, as well as to identify over-represented molecular pathways related to their pathogenesis.

Materials and Methods: Whole exome sequencing was performed on two DNA pools, one composed with DNA from 90 unspecified dementia patients and the other - a control pool with DNA from 100 age-synchronized healthy individuals. In total, 453748 variants were detected in the dementia patients' pool and 442765 in the control pool.

Results: Variants were selected so that that their population frequency is low, i.e. < 0.0025 in non-Finnish Europeans (gnomAD database) and correspondingly enriched in our dementia patients pool and control pools (frequency > 0.01). The gene list enrichment analysis platform *ToppGene* identified 37 prioritized molecular functions on our selected data, and the one involving the largest number of genes was *ribonucleotide binding*. Analysis by the Reactome platform on the genes involved in this molecular function showed that the immune system associated MAP kinase (MAPKs) activation pathway to be significantly enriched in dementia patients, but not in the control group.

Discussion: MAPKs are responsible for many cellular responses to cytokines and to external stress signals, and play an important role in regulating the production of inflammation mediators. Acknowledgment: KP-06-N33/5 from 13.12.2019 - National Science Fund of Bulgaria

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P09.044.A When two is one -a case of homozygous mutation in *STXBP1* gene as a result of single-parent disomy

Dorota Hoffman-Zacharska[1], *Anna Winczewska-Wiktor*[2], *Marta Smyk*[1], *Paulina Gorka-Skoczylas*[1], *Karolina Kanabus*[1], *Barbara Steinborn*[3]

[1]*Department of Medical Genetics, Institute of Mother and Child, Warsaw, Poland,* [2]*Department of Developmental Neurology, Poznan University of Medical Sciences, Poznan, Poland,* [3]*Department of Developmental Neurology, Poznan University of Medical Sciences, Warsaw, Poland.*

Introduction: Developmental and epileptic encephalopathy-4 (DEE4) is a condition starting in infancy and characterized by abnormal brain function (encephalopathy), intellectual disability accompanied often by recurrent seizures. This condition is caused by mutations in the *STXBP1* gene coding syntaxin-binding protein 1. The phenotypic spectrum of *STXBP1*-related disorders is broad, but the most cases result from heterozygous dominant, loss-of-function mutation of *de novo* character.

Materials and Methods: The family in which at the proband homozygous missense mutation in *STXBP1* gene was identified. Proband, 7 years old boy, was diagnosed with unclassified epileptic encephalopathy but with features of Lennox-Gastaut syndrome. Mutation was identified with the targeted NGS method - 49 EIEEs related genes panel. Sanger sequencing performed for proband and his parent confirmed *de novo* character of mutation. MLPA analysis showed no *STXBP1* gene deletions in patient nor in his parents. SNP microarray revealed in proband loss of heterozygosity along the entire chromosome 9, further analysis confirmed paternal origin of this chromosome.

Results: In this study, we identified *STXBP1* mutation p. Arg192Trp that have not been reported previously and was homozygous, what is rather unusual in the case of this gene. Detailed analysis revealed that mutation arose *de novo* on paternal chromosome, and its homozygosity is due to paternal uniparental disomy of the chromosome 9.

Conclusions: So far only one case of homozygous missense mutation has been described causing the Lennox-Gastaut syndrome. Here we report the new one, which may follow this same, unusual for *STXBP1*, gain-of-function effect on synaptic transmission.

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P09.045.B An attempt to identify gene variants responsible for neurological symptoms in children with adverse effects following immunization (AEFI)

Agnieszka Charzewska[1], *Dorota Hoffman-Zacharska*[1], *Iwona Terczyńska*[2], *Tomasz Mazurczak*[2], *Elżbieta Szczepanik*[2], *Alicja Karney*[3], *Tomasz Gambin*[1], *Karolina Kanabus*[1], *Renata Tataj*[1], *Jerzy Bal*[1]

[1]*Institute of Mother and Child, Department of Medical Genetics, Warsaw, Poland,* [2]*Institute of Mother and Child, Clinic of Neurology of Child and Adolescents, Warsaw, Poland,* [3]*Institute of Mother and Child, One Day Cllinic, Warsaw, Poland.*

Introduction: Vaccinations are one of the most significant achievements of medicine in modern times. Many catastrophic diseases, such as black pox or polio, have been eliminated due to vaccination. These diseases are completely unknown to modern parents, and therefore they question the validity of preventive vaccination, especially since none of the vaccines is completely free of side effects. Studies conducted on large populations show a lack of connection between vaccination and serious neurological symptoms, although there are isolated cases that indicate such a relationship. These reports on adverse effects following immunization (AEFI) reduce social confidence in vaccination, however their background may be rare genetic defects.

Materials and Methods: The aim of the presented study was verification the following research hypothesis: Neurological symptoms in children qualified as AEFI are related to the occurrence of pathogenic mutations in genes related to the development of the nervous system. To verify this hypothesis we performed whole exome sequencing (WES) in 22 patients with neurological AEFI.

Results: Our preliminary results suggest significant relationship between AEFI and the occurrence of mutations in genes associated with neurodevelopmental diseases. We identified pathogenic/VUS variants in 18/22 patients, 9 of them were acknowledged as definitely pathogenic due to parent examination. The mutated genes belonged to the group of genes associated with epilepsy syndromes/epileptic encephalopathy.

Conclusions: Preliminary results indicate that in many AEFI patients the vaccine can only trigger neurological symptoms that would have manifested anyway as a result of a pathogenic mutation in a gene engaged in neurodevelopment.

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P09.046.C Whole exome sequencing as instrument of molecular diagnosis in children with developmental and epileptic encephalopathies

Tatyana Kozhanova[1,2], *Svetlana Zhilina*[1,2], *Tatyana Mescheryakova*[1], *Karina Osipova*[1], *Sergey Ayvazyan*[1], *Nikolay Zavadenko*[2], *Andrey Prityko*[1,3]

[1]*Scientific and Practical Center of children medical care, Moscow, Russian Federation,* [2]*Pirogov Russian National Research Medical University, Moscow, Russian Federation,* [3]*Pirogov Russian National Research Medical University, Moscow, Russian Federation, Moscow, Russian Federation.*

Epilepsy is a complex disorder characterized by a predisposition to recurrent seizures that are caused by abnormal neuronal firing in the brain. Around 70—80% of all epileptic cases are caused by genetic mutations.

Materials and Methods: We examined 128 patients with developmental and epileptic encephalopathies. The clinical phenotyping, video-electroencephalography, computed and magnetic resonance imaging of the brain were carried out. All patients received informed consent for whole exome sequencing.

Results: The variants in genes were detected in 94/128 (73,4%) patients. No mutations have been identified in 34/128 (26,6%) patients. The variants in genes associated with early infantile epileptic encephalopathy were identified in 38/94 (40,4%) and variants in genes associated with mental retardation - in 16/94 (17%). The remaining patients had variants in genes associated with different types of epilepsy and neurodevelopmental disorders (25/94; 26,6%), tuberous sclerosis-2 (3/94; 3,2%), spastic paraplegia (3/94; 3,2%) and rare syndromes (3/94; 9,6%). The most commonly identified genes were *SCN1A - 5, CACNA1A - 3, GABBR2 - 2, GRIN1 - 2, GRIN2B - 2, TSC2 - 3, SYNGAP1 - 2, SPTAN1 - 2, PCDH19 - 2, TRIO - 2, CHD2 -2, FGF12 - 2, KCNQ2 - 2, MECP2 - 2*.

Conclusion: Genetic testing has become a first line test in epilepsy. Our study suggests that WES is an effective diagnostic tool and encourage the interaction between an epileptologist and geneticist. Genetic diagnosis can help to consolidate the clinical diagnosis, to facilitate phenotypic expansion, and to influence

treatment and management options for seizure control in our patients.

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P09.047.D *PIGG* variant pathogenicity assessment reveals novel features within nineteen families

Camille Tremblay-Laganiere[1], *Reza Maroofian*[2], *Thi T. M. Nguyen*[1], *Ehsan Ghayoor Karimiani*[3], *Salman Kirmani*[4], *Fizza Akbar*[4], *Shahnaz Ibrahim*[4], *Bushra Afroze*[4], *Mohammad Doosti*[3], *Farah Ashrafzadeh*[5], *Meisam Babaei*[6], *Stephanie Efthymiou*[2], *Tipu Sultan*[7], *Roger L. Ladda*[8], *Heather M. McLaughlin*[9], *Rebecca Truty*[9], *Sonal Mahida*[10], *Julie S. Cohen*[10], *Kristin Baranano*[10], *Fatima Y. Ismail*[11], *Millan S. Patel*[12], *Anna Lehman*[12], *Andrew C. Edmondson*[13], *Amanda Nagy*[14], *Melissa A. Walker*[14], *Saadet Mercimek-Andrews*[15], *Yuta Maki*[16], *Rani Sachdev*[17], *Rebecca Macintosh*[17], *Elizabeth E. Palmer*[17], *Grazia M.S. Mancini*[18], *Tahsin S. Barakat*[18], *Robert Steinfeld*[19], *Christina T. Rüsch*[19], *Georg M. Stettner*[19], *Matias Wagner*[20], *Saskia B. Wortmann*[21], *Usha Kini*[22], *Angela F. Brady*[23], *Karen L. Stals*[24], *Naila Ismayilova*[25], *Sian Ellard*[24], *Danilo Bernardo*[26], *Kimberly Nugent*[27], *Scott D. McLean*[27], *Stylianos E. Antonarakis*[28], *Henry Houlden*[2], *Taroh Kinoshita*[16], *Philippe M. Campeau*[1], *Yoshiko Murakami*[16]

[1]*University of Montreal, Montreal, QC, Canada,* [2]*UCL Queen Square Institute of Neurology and The National Hospital for Neurology and Neurosurgery, London, United Kingdom,* [3]*Next Generation Genetic Polyclinic, Mashhad, Iran, Islamic Republic of,* [4]*Aga Khan University, Karachi, Pakistan,* [5]*Mashhad University of Medical Sciences, Mashhad, Iran, Islamic Republic of,* [6]*North Khorasan University of Medical Sciences, Bojnurd, Iran, Islamic Republic of,* [7]*The Children's Hospital Lahore, Lahore, Pakistan,* [8]*Milton S Hershey Medical Centre, Hershey, PA, USA,* [9]*Invitae, San Francisco, CA, USA,* [10]*Kennedy Krieger Institute, Baltimore, MD, USA,* [11]*Johns Hopkins University School of Medicine, Baltimore, MD, USA,* [12]*University of British Columbia, Vancouver, BC, Canada,* [13]*Children's Hospital of Philadelphia, Philadelphia, PA, USA,* [14]*Massachusetts General Hospital, Boston, MA, USA,* [15]*University of Toronto, Toronto, ON, Canada,* [16]*Osaka University, Osaka, Japan,* [17]*Sydney Children's Hospital, Sydney, Australia,* [18]*Erasmus MC University Medical Center, Rotterdam, Netherlands,* [19]*University Children's Hospital Zurich, Zurich, Switzerland,* [20]*Technical University Munich, Munich, Germany,* [21]*Paracelsus Medical School, Salzburg, Austria,* [22]*Churchill Hospital, Oxford, United Kingdom,* [23]*Northwick Park Hospital, Harrow, United Kingdom,* [24]*Royal Devon and Exeter NHS Foundation Trust, Exeter, United Kingdom,* [25]*Chelsea and Westminster Hospital, London, United Kingdom,* [26]*University of California San Francisco, San Francisco, CA, USA,* [27]*The Children's Hospital of San Antonio, San Antonio, TX, USA,* [28]*University of Geneva Medical School, Geneva, Switzerland.*

Purpose: Phosphatidylinositol Glycan Anchor Biosynthesis, class G (PIGG) is an ethanolamine phosphate transferase catalyzing the modification of the second mannose of glycosylphosphatidylinositol (GPI). GPIs serve as anchors on the cell membrane by linking over 150 surface proteins called GPI anchored proteins (GPI-APs) on their third mannose. Pathogenic variants in genes involved in the biosynthesis of GPI cause inherited GPI deficiency (IGD) which still needs to be further characterized.

Methods: We describe twenty-two individuals from nineteen new unrelated families with bi-allelic variants in *PIGG*. We analyzed GPI-AP surface levels on granulocytes and fibroblasts for three and two individuals, respectively. We demonstrated enzymatic activity defects for *PIGG* variants in vitro in a *PIGG*/*PIGO* double knockout system.

Results: Phenotypic analysis of reported individuals reveals novel PIGG deficiency associated features. All tested GPI-APs were unchanged on granulocytes whereas FLAER and CD73 levels in fibroblasts were decreased. In addition to common symptoms such as hypotonia, intellectual disability/developmental delay, and seizures, individuals with PIGG variants of null or severely decreased activity showed cerebellar abnormalities, neurological manifestations, and mitochondrial dysfunction, a feature increasingly recognized in IGDs. Individuals with mildly decreased activity variants showed autism spectrum disorder.

Conclusion: This in vitro system is a useful method to validate the pathogenicity of new variants in *PIGG* and to study PIGG physiological functions. Recent work using this system has identified a new subset of PIGG-dependent GPI-APs with an alternative bridging on the second mannose. Reduced levels of specific PIGG-dependent GPI-APs might explain the phenotype observed in individuals with PIGG-deficiency.

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P09.048.A *SCN9A* gene variants do not cause monogenic epilepsy in humans

James D. R. Fasham[1], *Joseph S. Leslie*[1], *Jamie W. Harrison*[1], *James Deline*[2], *Katie B. Williams*[3], *Ashley Kuhl*[3], *Jessica Scott Schwoerer*[3], *Harold E. Cross*[4], *Andrew H. Crosby*[1], *Emma L. Baple*[1]

[1]*University of Exeter, Exeter, United Kingdom,* [2]*Center for Special Children, La Farge Medical Clinic-VMH, La Farge, WI, USA,* [3]*Department of Pediatrics, University of Wisconsin, Madison, WI, USA,* [4]*Department of Ophthalmology, University of Arizona College of Medicine, Tucson, AZ, USA.*

Many studies demonstrate the clinical utility and importance of epilepsy gene panel testing to confirm the specific aetiology of disease, enable appropriate therapeutic interventions, and inform accurate family counselling. Previously, *SCN9A* gene variants, in particular the c.1921A>T p.(Asn641Tyr) substitution, have been defined as likely autosomal dominant causes of febrile seizures plus and other monogenic seizure phenotypes indistinguishable from those associated with *SCN1A*. This led to the inclusion of *SCN9A* on epilepsy gene panels globally.

In the Amish and other community settings both pathogenic and benign gene variants may become enriched, enabling their clinical interpretation. Here we present serendipitous genetic findings that identify *SCN9A* c.1921A>T p.(Asn641Tyr) at high frequency among the Amish, in the absence of seizure phenotypes. This, alongside review of published cases and UK Biobank findings, clearly refutes an association of *SCN9A* with epilepsy.

Given this, the presence of *SCN9A* on gene testing panels and its currently widely accepted status as an epilepsy disease gene, clearly present a substantial misdiagnosis risk to patients. This is of particular concern where a precise epilepsy molecular diagnosis

informs drug choice and misdiagnosis may have devastating and lethal consequences. Our findings highlight the importance of ClinGen and other expert groups, and urge reappraisal of the evidence regarding *SCN9A* in monogenic seizure phenotypes, to mitigate potential harms. Further, our studies demonstrate the importance of genomic community studies worldwide, where due to ancestral genetic bottleneck events enrichment of otherwise rare variants allows improved interpretation of pathogenicity.

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P09.049.B *In silico* association of the seizures phenotype to iron-induced non-apoptotic cell death

Alejandro Cisterna García[1], *Irene Díez García-Prieto*[2], *Paolo Maietta*[2], *Sara Álvarez de Andrés*[2], *Álvaro Sánchez-Ferrer*[1], *Juan A. Botía*[1]

[1]*Universidad de Murcia, Murcia, Spain,* [2]*NIMGenetics, Madrid, Spain.*

Epileptic seizures represent a central phenotype within epilepsy. We wanted to expand our knowledge about the phenotypes associated with genes linked to seizures. We obtained the genes associated with Seizure (Human Phenotype Ontology HP:0001250) and performed a phenotype enrichment analysis with PhenoExamWeb. We observe that the genes associated with seizures are also linked to Labile Iron (CRB:0000004 ; P = 3.15x10-12) and Peroxidized Lipids (CRB:0000007; P = 2.15x10-19) using CRISPR-Brain database within PhenoExamWeb. Terms that are not only relevant in epilepsy and glutamatergic neurons, but are also associated with ferroptosis, which is a novel form of non-apoptotic regulated cell death attributed to severe lipid peroxidation caused by ROS production and iron overload. Although ferroptosis has recently been studied in epilepsy, the genetics underlying this process remain unclear. The 49 genes shared by the three annotation terms suggest a possible interesting novel ferroptosis-seizure relationship (Fisher´s Exact for the overlap P = 5.95x10-12). Those genes are linked to Epilepsy (C0014544; P = 0.012), Leigh Disease and Mitochondrial Complex I Deficiency (C1838979; P = 1.9x10-12). They are highly expressed in brain tissues according to GTExV8, preferentially expressed in Pyramidal neurons (P = 0.0104) among other mice brain cells according to EWCE. We also observe that some of our genes and GPX4 are in the same co-expression network module within cortex and hippocampus tissue networks according to CoExp Web. These evidences may suggest a role for ferroptosis in epilepsy and also point out this process to certain genes.Fundación Séneca and NIMGenetics.

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P09.050.C Genetic studies in epilepsy. Experience of a third level pediatric hospital

Nelmar Valentina Ortiz Cabrera, *Bárbara Fernández Garoz, Elena González Alguacil, María Jiménez Legido, Ruth Camila Púa Torrejón, Víctor Soto Insuga, Teresa Moreno Cantero, Beatriz Bernardino Cuesta, Anna Duat Rodriguez, Verónica Cantarín Extrémera, María Luz Ruiz-Falcó Rojas, Juan José García Peñas*

Hospital Infantil Universitario Niño Jesús, Madrid, Spain.

Epileptic encephalopathy (EE) causes severe cognitive and behavioural issues, shows variable expressivity and could be progressive. EE may present alone, accompanied by a cortical malformation disorder or taking part of a syndromic entity.

Aim: We collected data from a third level children´s hospital specialized in treating EE to evaluate the diagnostic yield of genetic studies based on next generation sequencing.

Patients: We collected data of patients whose primary diagnosis was epilepsy or EE, referred to the Clinical Genetic clinic by the Neurology department for study between June 2018 and December 2020. We separated patients in different phenotypic clusters. Cluster 1: syndromic epileptic encephalopathy (HP:0200134), ASD (HP:0000729, HP:0000717), Rett-like phenotype, spastic paraplegia (HP:0100021) or suspected mitochondrial disease (HP:0003287). Cluster 2: cortical developmental disorder and/or focal epilepsy. Cluster 3: epilepsy without intellectual disability including febrile seizures. Genes analysed were selected depending on the phenotypic cluster.

Results: 137 patients were studied. Total diagnostic yield was 25% with 17% of non-conclusive results due to variants of unknown significance. Analysing the data using clusters we found that Cluster 1 and 2 had the maximum yield of 32 and 23%, respectively. Pathogenic variants in *CDKL5* were present in 3 non-related individuals of cluster 1, pathogenic variants in *DEPDC5* were present in 3 non-related individuals of cluster 2.

Discussion and conclusion: The study of Epileptic encephalopathy using next generation sequencing approach has a good diagnostic yield. Whereas the study of epilepsy without intellectual disability has a low diagnostic yield, except if there is a clear inheritance pattern.

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P09.051.D A novel disorder causing fetal cerebral hemorrhages associated to a homozygous mutation of the *ESAM* gene

Jessica Hernandez Rodriguez[1], *A Ruiz*[2], *Elena Miravet*[2], *Victor Asensio Landa*[3], *Rosa Martorell-Riera*[2], *Maria Carmen Vidal Lampurdanes*[2], *Laura Torres-Juan*[3], *Iciar Martinez-Lopez*[3], *Emilia Amengual-Cladera*[3], *Cristofol Vives Bauza*[3], ***DAMIAN HEINE SUÑER***[3]

[1]*Institut d'Investigació Sanitaria de Palma (IDISBA), Palma, Spain,* [2]*Hospital Universitari Son Espases, Palma, Spain,* [3]*Hospital Universitari Son Espases/Institut d'Investigació Sanitaria de Palma (IDISBA), Palma, Spain.*

The stability and permeability of the blood vessel wall relies on endothelial junctions, which are formed and regulated by cell adhesion molecules. One of them is ESAM (endothelial adhesion molecule), structurally related to JAM proteins, and all form part of the blood-brain barrier. Furthermore, a homozygous mutation in JAM3 has been reported to cause hemorrhagic destruction of the brain. However, up to now ESAM has not been associated to any disease. In our study, a brother and sister from non-consanguineous parents suffered severe prenatal cerebral

hemorrhages. As a result both siblings, now 2 and 4 years old, show spastic tetraparesis, seizures, hydrocephalus, dysphagia, respiratory problems and cortical blindness. Whole exome sequencing (WES) analysis was oriented towards known genes associated to hereditary bleeding or brain disorders but no significant variant could be found. After this, we performed analysis for runs of homozygosity shared between brother and sister, and found a single 2,3 Mb homozygous block on chromosome 11q24.1 which contained 46 homozygous variants in 19 genes, of which only 1 was likely pathogenic: a previously not described frameshift mutation in exon 3 of the *ESAM* gene (NM_138961.3; c.287delC/ p.Pro96fs). Mutation analysis of parents and grandparents found both parents and the paternal and maternal grandmothers to be carriers but no evidence of consanguinity as they come from very distant parts of Spain. RNA expression and ancestry studies will be presented.

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P09.052.A Rare pathogenic variants in genes of glutamatergic neurotransmission pathway segregate with schizophrenia in Pakistani families

*Ambrin Fatima[1,2,3], **Uzma Abdullah**[4,3,5], Muhammad Farooq[6,2], Zafar Ali[2], Yuan Mang[2], Mana M. Mehrjouy[2], Niels Tommerup[2], Shahid M Baig[3,1]*

[1]Department of Biological and Biomedical Sciences,The Aga Khan University, Karachi, Pakistan, [2]Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark, [3]National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan, [4]University Institute of Biochemistry and Biotechnology (UIBB).PMAS-Arid Agriculture University, Rawalpindi, Pakistan, [5]University of Copenhagen, Copenhagen, Denmark, [6]Department of Biotechnology, Institute of Biochemistry, Biotechnology and Bioinformatics (IBBB), The Islamia University of Bahawalpur, Bahawalpur, Pakistan.

Introduction: Schizophrenia is a disabling neuropsychiatric disorder of adulthood onset with high heritability. World-wide collaborations have identified association of ~270 common loci, with small individual effect and hence weak clinical implications. Recent technological feasibility of exome sequencing is enabling identification of rare variants of high penetrance, that refine previous findings and improve risk assessment and prognosis.

Material and Methods: We recruited two multiplex Pakistani families, having 11 patients and 19 normal individuals in three generations. We performed genome-wide SNP genotyping, next generation mate pair and whole exome sequencing to unveil genetic component. Candidate variants were screened in unrelated cohorts of 508 cases, 300 controls and fifteen families (with 51 affected and 47 normal individuals) of Pakistani origin. Structural impact of substituted residues was assessed through *in silico* modelling using iTASSER.

Results: In one family, we identified a rare novel microduplication (5q14.1_q14.2) encompassing critical genes involved in glutamate signaling such as *CMYA5, HOMER*, RasGRF2. Second family segregates two rare, predicted pathogenic variants NM_001134407.3 (*GRIN2A)*: c.3505C>T, (p.R1169W) and NM_001010848.4 (NRG3):c.1951G>A,(p.E651K). These genes encode for parts of AMPA and NMDA receptors of glutamatergic neurotransmission respectively and the variants are predicted to compromise protein function by destabilizing their structures. The variants were absent in aforementioned cohorts.

Conclusion: Our findings suggest that rare, highly penetrant variants of genes involved in glutamatergic neurotransmission are contributing to etiology of schizophrenia in these families. It also highlights that genetic investigations of multiplex, multigenerational families with could be a powerful approach to identify rare genetic variants involved in complex disorders.

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P09.054.C FKBP5 in First-Episode Psychosis: Insights from a Greek population sample

***Daniela Theodoridou**[1], Alexandra Polyzou[2], Angeliki Maria Vlaikou[3,4], Michaela D. Filiou[3,4], George Leondaritis[2], Petros Petrikis[5], Marika Syrrou[1]*

[1]Laboratory of Biology, Faculty of Medicine, School of Health Sciences, University of Ioannina, Ioannina, Greece, [2]Department of Pharmacology, Faculty of Medicine, School of Health Sciences, University of Ioannina, Ioannina, Greece, [3]Laboratory of Biochemistry, Department of Biological Applications and Technology, School of Health Sciences, University of Ioannina, Ioannina, Greece, [4]Biomedical Research Division, Institute of Molecular Biology and Biotechnology, Foundation of Research and Technology Hellas (IMBB-FORTH), Ioannina, Greece, [5]Department of Psychiatry, Faculty of Medicine, School of Health Sciences, University of Ioannina, Ioannina, Greece.

Introduction: The hypothalamus-pituitary-adrenal axis mediates the neuroendocrine response to stress. FKBP5 is a co-chaperone of the cortisol-bound glucocorticoid receptor. SNPs in the *FKBP5* locus may affect its expression levels and have been associated with psychopathology. Our objective is to investigate the distribution of SNP rs1360780 and FKBP5 mRNA and protein expression in individuals with First-Episode Psychosis (FEP) at two time points: at the onset of FEP and after treatment with second generation antipsychotics (SGAs).

Materials and Methods: Upon admission, 21 individuals were diagnosed with the Positive And Negative Symptom Scale (PANSS) and whole blood was extracted. rs1360780 (C->T) was genotyped using Taqman SNP genotyping assay. Total RNA and protein content were extracted from PBMCs, both upon admission and after monotherapy with SGAs. FKBP5 expression levels were assessed with RT-qPCR and Western Blot. mRNA levels were normalized against the 18s rRNA and protein levels against the GAPDH reference genes, respectively. Statistical analysis was performed by GraphPad Prism 8.

Results: In our sample ($N = 21$), 80% of males and 50% of females are homozygous for the C (protective) allele. The rest carry at least one T (risk) allele for the rs1360780. *FKBP5* mRNA levels decrease after antipsychotic treatment ($^{**}p = 0.0095$). The FKBP5 protein levels in a subgroup of individuals ($N = 12$) were assessed before and after treatment and were not significantly different ($p = 0.1763$).

Conclusions: We observe altered mRNA but no protein *FKBP5* expression level alterations. Further studies and larger population data will shed light on the putative role of SGAs on FKBP5 expression.

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P09.055.D Identifying lipid metabolism genes with a potential role in the pathogenesis of Frontotemporal dementia through pool exome sequencing

Mihail Ganev[1], *Dimitar Serbezov*[1], *Lubomir Balabanski*[1,2], *Radoslava Vazharova*[2,3], *Sena Karachanak-Yankova*[1,4], *Olga Antonova*[1], *Dragomira Nikolova*[1], *Marta Mihaylova*[1], *Rada Staneva*[1], *Viktoria Spasova*[1], *Vera Damyanova*[1], *Desislava Nesheva*[1], *Zora Hammoudeh*[1], *Blaga Rukova*[1], *Savina Hadjidekova*[1], *Shima Mehrabian*[5], *Maria Petrova*[5], *Diana Belezhanska*[5], *Lachezar Traykov*[5], *Draga Toncheva*[1,2]

[1]*Department of Medical Genetics, Medical University of Sofia, Sofia, Bulgaria,* [2]*Gynecology and assisted reproduction hospital "Malinov", Sofia, Bulgaria,* [3]*Department of Biology, Medical genetics and Microbiology, Faculty of Medicine, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria,* [4]*Department of Genetics, Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria,* [5]*Depatment of Neurology, UH "Alexandrovska", Medical University of Sofia, Sofia, Bulgaria.*

Introduction: Molecular pathogenesis of Frontotemporal dementia (FTD) is associated with intracellular accumulation of proteins in central nervous system. However, it is unknown whether genetically determined lipid disturbances could also be a contributing factor (like the *ApoE* polymorphisms in Alzheimer's disease). This study aims to identify dyslipidemia-associated genes with a potential for further investigation of a pathogenic role in FTD.

Materials & Methods: Whole-exome sequencing was performed on two DNA pools set up of frontotemporal dementia (FTD) patients (n = 66) and healthy individuals (n = 100), respectively. After quality filtering, the 48,455 annotated rare genetic variants detected in the FTD pool were used to compile a gene list. The list was subjected to functional enrichment analysis yielding 313 genes from the Gene Ontology "lipid binding" molecular function group. As a last step, a pathway prioritizing of the "lipid binding" genes was carried out. The same workflow has been applied to the data from the healthy individuals' pool.

Results: Analysis of the FTD pool data led to the prioritization of 9 pathways with a total of 18 genes associated with plasma lipoprotein assembly, remodeling, and clearance. Healthy individuals' pool data did not yield overrepresented rare variant genes from the "lipid binding" molecular function group. Results indicate a possible role of some lipid metabolism genes in the pathogenesis of FTD.

Conclusion: The analysis of whole-exome pool sequencing data of FTD patients and heathy individuals has identified 18 lipid metabolism-associated genes potentially associated with FTD pathogenesis. **Acknowledgment:** KP-06-N33/5 from 13.12.2019 - National Science Fund of Bulgaria.

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P09.056.A Topological mapping of variant-intolerant domains in SCN1A using a novel functional modeling platform

Matteo Vatta, *Dianalee McKnight, Karen Ouyang, Ana Morales, Swaroop Aradhya*

Invitae, San Francisco, CA, USA.

Introduction: The clinical classification of novel missense variants is challenging due to insufficient evidence, often leaving them categorized as variants of uncertain significance (VUS). To generate additional evidence to better interpret the effects of missense VUS, we developed a gene-specific functional modeling platform (FMP) evaluating DNA sequence conservation, biophysical, structural, cellular, and spatial relationships within an observed missense change setting. Here, we evaluate the accuracy of FMP for classifying variants in the SCN1A-encoded NaV1.1 ion channel previously associated with severe epilepsy syndromes.

Methods: FMP data on 619 missense SCN1A variants predicted to be deleterious (P) or tolerated (B) were investigated for their topological enrichment to identify variant-intolerant protein domains.

Results: FMP predicted 468/619 (75.6%) variants as P and 151/619 (24.4%) as B. Of the 468 P variants, 150 (32%) resided in the cytoplasmic region, including a C-terminal cluster within the CaM-binding domain (aa 1915-1944), 119 (25.4%) in the transmembrane, 29 (6.2%) in the S4-voltage sensor, 45 (9.7%) in the "pore", and 125 (26.7%) in the extracellular domains. FMP predicted 107 (70.9%) B variants in the cytoplasmic, 10 (6.6%) in transmembrane, and 34 (22.5%) in extracellular domains. No B-predicted variants resided in the S4-voltage sensor, "pore", or CaM-binding domains, suggesting these regions are particularly variant-intolerant.

Conclusions: These data demonstrate the predictive accuracy of FMP in NaV1.1 in identifying specific functional domains apparently intolerant to genetic variability, establishing its utility in supporting clinical variant interpretation in germline genetic testing.

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P09.057.B Screening for the *FMR1* premutation in Greek patients with late-onset cerebellar ataxia

Maria Seferiadi[1], *Chrisoula Kartanou*[1], *Christalena Sofocleous*[2,3], *Joanne Traeger-Synodinos*[2], *Georgia Karadima*[1], *George Koutsis*[1]

[1]*Neurogenetics Unit, 1st Department of Neurology, School of Medicine, Eginition Hospital, National and Kapodistrian University of Athens, Athens, Greece,* [2]*Laboratory of Medical Genetics, School of Medicine, University of Athens, "Aghia Sophia" Children's Hospital, Athens, Greece,* [3]*Research University Institute for the Study of Genetic and Malignant Diseases of Childhood, "Aghia Sophia" Children's Hospital, Athens, Greece.*

Fragile X-associated tremor/ataxia syndrome (FXTAS) is a late-onset, X-linked, neurodegenerative disorder that occurs in premutation carriers of the *FMR1* gene. Although core motor features include gait ataxia and action tremor, some patients demonstrate parkinsonism, cognitive deficits and peripheral neuropathy. Consequently, FXTAS is often misdiagnosed as spinocerebellar ataxia (SCA) or Parkinson's disease (PD). In Greek patients with ataxia, although the most common SCAs have been studied by our group, the occurrence of FXTAS has not been previously investigated. Herein, we sought to investigate the frequency, genotypic and phenotypic profile of FXTAS in Greek patients with late-onset cerebellar ataxia.From a cohort of 454 ataxia cases (SCA1, 2, 3, 6, 7 negative), 92 index patients were selected, clinically characterized by ataxia (100%), tremor (19%), polyneuropathy (14%), parkinsonism (9.8%), and cognitive decline (8.7%). All cases had no male-to male transmission. Genotyping was

performed using fragment analysis by capillary electrophoresis. We detected two *FMR1* premutation carriers (2.2%), well within the range reported by multiple studies in ataxic cohorts (0-4.1%), and higher than other, movement disorder, cohorts (<1%). Both patients had cerebellar ataxia and neuropathy. One patient also had mild parkinsonism and cognitive impairment, and the other with pyramidal signs. We conclude that *FMR1* premutations are not rare in Greek patients with late-onset cerebellar ataxia. In light of the above, molecular screening for FXTAS should be considered in SCA panel negative hereditary ataxia cases with supportive clinical features. Our study highlights the importance of genetic testing in the differential diagnosis and early management of FXTAS.

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P09.058.C Characterization of SUMO2/3 protein levels in Fragile X-associated tremor/ataxia syndrome patients

Laia Rodriguez-Revenga[1,2], *Tamara Barcos*[1], *Emma Peruga*[1], *Laura Molina-Porcel*[3,4], *Maribel Alvarez-Mora*[1,2]

[1]*Hospital Cinic, Barcelona, Spain,* [2]*CIBER of Rare Diseases, Instiuto de Salud Carlos III, Barcelona, Spain,* [3]*Alzheimer's disease and other cognitive disorders unit. Neurology Service, Hospital Clínic, Institut d'Investigacions Biomediques August Pi i Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain,* [4]*Neurological Tissue Bank,Biobanc-Hospital Clínic-IDIBAPS, Barcelona, Spain.*

Introduction: Fragile X-associated tremor/ataxia syndrome (FXTAS) is a late-onset neurodegenerative disorder with reduced penetrance that appears in adult *FMR1* premutation carriers (55-200 CGGs). The neuropathological hallmark of FXTAS consists of presence of ubiquitin-positive nuclear inclusions that are broadly distributed throughout the brain. The small ubiquitin-related modifier 2/3 (SUMO 2/3), which is an element of the cellular response to environmental stress, has been recently described as one of the most highly abundant proteins in FXTAS inclusions. Since bioenergetic collapse leading to cellular stress has been well reported in FXTAS patients, we aimed to characterize SUMO2/3 protein levels in FXTAS patients.

Material and Methods: Fibroblasts cultures from FXTAS patients were used to quantify SUMO2/3 protein levels by western blot analysis. Immunohistochemistry experiments were also performed in *postmortem* brain samples from FXTAS patients.

Results: SUMO2/3 quantification in whole fibroblasts culture lysates did not revealed significant differences between FXATS and control samples. However, immunohistochemistry analysis revealed positive SUMO2/3 staining in intranuclear inclusions of FXTAS *postmortem* brain samples.

Conclusions: although the pathogenic mechanisms inducing neurodengeneration and the development of inclusions in *FMR1* premutation carriers are unknown, our results support a role of SUMO2/3 in the process. Acknowledgements: Work supported by the Instituto de Salud Carlos III (PI17/01067), co-financed by Fondo Europeo de Desarrollo Regional (FEDER) "una manera de hacer Europa" and AGAUR (2017 SGR1134). The CIBERER is an initiative of the Instituto de Salud Carlos III. We thank brain donors and relatives for generous donation and the Neurological Tissue Bank of the Biobanc-Hospital Clinic-IDIBAPS.

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P09.059.D Structural mapping of *GABRB3* variants reveal correlations between genotype and phenotype

Katrine M. Johannesen[1,2], *Sumaiya Iqbal*[3,4], *Milena Guazzi*[5,1], *Nazanin A. Mohammadi*[1,2], *Eduardo Perez*[6], *Dennis Lal*[7,8,9,10], *Elise Schaefer*[11], *Anne De Saint Martin*[12], *Marie Therese Abiwarde*[12], *Amy McTague*[13], *Roser Pons*[14], *Amelie Piton*[15], *Manju Kurian*[16], *Marie Deprez*[17], *Liesbeth de Waele*[18,19], *Eva Brilstra*[20], *Nienke E. Verbeek*[20], *Marjan van Kempen van Kempen*[20], *Gerhard Visser*[21], *Hilde M. H. Braakman*[22], *Martin Haeusler*[23], *Miriam Elbracht*[24], *David Sternman*[25], *Ulvi Vaher*[26], *Thomas Smol*[27], *Joanna Kennedy*[28], *Karl Martin Klein*[29], *Billie Au*[30], *Kimberly Smyth*[31], *Thomas Morgan*[32], *Malin Dewenter*[33], *Argirios Dinopoulos*[34], *Damien Lederer*[35], *Vivian Liao*[36], *Philip K. Ahring*[36], *Rikke S. Møller*[1,2], *Elena Gardella*[37,2]

[1]*Department of Epilepsy Genetics and Personalized Treatment, The Danish Epilepsy Centre, Dianalund, Denmark,* [2]*Institute for Regional Health Services, University of Southern Denmark, Odense, Denmark,* [3]*Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, Cambridge, MA, USA,* [4]*Center for the Development of Therapeutics, Broad Institute of MIT and Harvard, Cambridge, MA, USA,* [5]*Department of Medicine, University of Genoa, Genoa, Italy,* [6]*Center for Genetics and Genomics Instituto de Ciencias e Innovación en Medicina (ICIM) Universidad del Desarrollo, Santiago, Chile,* [7]*Genomic Medicine Institute, Lerner Research Institute, Cleveland Clinic, Cleveland, OH, USA,* [8]*Epilepsy Center, Neurological Institute, Cleveland Clinic, Cleveland, OH, USA,* [9]*Stanley Center for Psychiatric Research, Broad Institute of Harvard and M.I.T, Cambridge, MA, USA,* [10]*Cologne Center for Genomics (CCG), University of Cologne, Cologne, Germany,* [11]*Service de Génétique Médicale, CMCO-SIHCUS, Hôpital de Hautepierre, Strassbourg, France,* [12]*Department of Pediatric Neurology, Strasbourg University Hospital, Strassbourg, France,* [13]*Molecular Neurosciences, Developmental Neurosciences Programme, UCL Great Ormond Street Institute of Child Health, London, United Kingdom,* [14]*First Department of Pediatrics, "I Agia Sofia" Children Hospital, National & Kapodistrian University of Athens, Athens, Greece,* [15]*Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France,* [16]*Institute of Child Health, Great Ormond Street Hospital for Children, London, United Kingdom,* [17]*Université Côte d'Azur, CNRS, IPMC, Sophia-Antipolis, France,* [18]*Department of Development and Regeneration, Campus Kulak Kortrijk, Kortrijk, Netherlands,* [19]*Department of Paediatric Neurology, University Hospitals Leuven, Leuven, Netherlands,* [20]*Department of Genetics, University Medical Center Utrecht, Utrecht University, Utrecht, Netherlands,* [21]*Stichting Epilepsie Instellingen Nederland (SEIN), Hoofddorp, Netherlands,* [22]*Department ofAcademic Center for Epileptology Kempenhaeghe/ Maastricht University Medical Center Epilepsy Genetics and Personalized Treatment, The Danish Epilepsy Centre, Heeze, Netherlands,* [23]*Institute of Evolutionary Medicine, University of Zurich, Zurich, Switzerland,* [24]*Institute of Human Genetics, Medical Faculty, RWTH Aachen University, Aachen, Germany,* [25]*Division of Neurology Lincoln Medical and Mental Health Center, Bronx, NY, USA,* [26]*Children's Clinic of Tartu University Hospital, Tartu, Estonia,* [27]*Institut de Genetique Medicale, CHRU Lille, Universite de Lille, Lille, France,* [28]*Clinical Genetics Department, University Hospitals Bristol NHS Foundation Trust, St Michael's Hospital, Bristol, United Kingdom,* [29]*Epilepsy Centre Frankfurt Rhein-Main, University Hospital Frankfurt, University of Frankfurt, Frankfurt, Germany,* [30]*Department of Medical Genetics, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada,* [31]*Department of Pediatrics, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada,* [32]*Precision Medicine/Genetic Testing Stewardship Program, Nemours A. I. duPont Hospital for Children Precision Medicine/Genetic Testing Stewardship Program, Wilmington, DE, USA,* [33]*Universitätsmedizin Johannes Gutenberg-University Mainz Institut für Humangenetik, Mainz, Germany,* [34]*Attiko University Hospital, University of Athens, 3rd Dept of Pediatrics, Haidari, Greece,* [35]*Centre de Génétique Humaine, Institut de Pathologie et de Génétique, Charleroi, Belgium,* [36]*Brain and Mind Centre, School of Pharmacy, Faculty of Medicine and Health, The University of Sydney, Sydney, Australia,* [37]*Department of Clinical neurophysiology, the Danish Epilepsy Centre, Dianalund, Denmark.*

Objective: It has previously been shown that pathogenic variants in the *GABRB3* gene increase seizure susceptibility and lead to a broad phenotypic spectrum ranging from severe developmental and epileptic encephalopathies to milder epilepsy syndromes such as generalized epilepsy with febrile seizures + and childhood absence epilepsy. With a cohort of 76 published an unpublished patients, we aimed to highlight possible correlations between phenotype and genotype in *GABRB3*.

Material and Methods: Through an international collaboration and literature review, we analyzed electro-clinical data of patients with variants in *GABRB3*. All variants was mapped to the 3D structure of the *GABRB3* subunit.

Results: 76 patients with pathogenic or likely pathogenic *GABRB3* variants, including 24 previously unpublished patients, were included in the study. Clinical phenotype correlated with structural location: Patients with variants in the extracellular domain had febrile seizure, myoclonic seizures and epileptic spams with onset at ten months, while patients with variants in the transmembrane domain had focal seizures with or without secondary generalization with onset at six months.

Conclusion: Our results suggest a genotype/phenotype correlation, with variants in the extracellular domain causing milder phenotypes with generalized epilepsy and variants in the transmembrane domains causing more severe phenotypes with early-onset focal epilepsy. Whether or not functional difference also plays a part in the phenotypic differences will require further research. These correlations are of importance as we stand on the brink of precision medicine in the genetic epilepsies. KMJ was funded by the Lundbeck Foundation (R324-2019-1083)

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P09.061.B Elevated expression of *SLC6A4* encoding the serotonin transporter (SERT) in Gilles de la Tourette syndrome

Amanda M. Levy[1], *Mathis Hildonen*[1], *Christina Dahl*[2], *Victoria A. Bjerregaard*[1], *Lisbeth Birk Møller*[1,3], *Per Guldberg*[2,4], *Nanette M. Debes*[5], *Zeynep Tümer*[1,6]

[1]*Kennedy Center, Department of Clinical Genetics, Copenhagen University Hospital, Rigshospitalet, Glostrup, Denmark,* [2]*Danish Cancer Society Research Center, Copenhagen, Denmark,* [3]*Institute for Nature, Systems and Models, Roskilde University Center, Roskilde, Denmark,* [4]*Department of Cancer and Inflammation Research, Institute for Molecular Medicine, University of Southern Denmark, Odense, Denmark,* [5]*Tourette Clinics, Department of Paediatrics, Copenhagen University Hospital, Herlev, Denmark,* [6]*Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark.*

Introduction: Gilles de la Tourette syndrome (GTS) is a complex neurodevelopmental disorder characterized by motor and vocal tics. Most of the GTS individuals have comorbid diagnoses, of which obsessive-compulsive disorder (OCD) and attention deficit-hyperactivity disorder (ADHD) are the most common. Several neurotransmitter systems have been implicated in disease pathogenesis, and amongst these, the dopaminergic and the serotonergic pathways are the most widely studied. In this study, we aimed to investigate whether the serotonin transporter gene (*SLC6A4*) was differentially expressed among GTS individuals, and whether DNA variants (5-HTTLPR, rs25531 and rs25532) or promoter methylation was associated with GTS phenotype, or *SLC6A4* expression.

Methods: DNA from peripheral blood samples was obtained from 72 GTS individuals and 87 controls, and RNA from 56 GTS individuals and 36 controls. All individuals were genotyped by PCR followed by sanger sequencing, and *SLC6A4* expression was quantified using RT-qPCR. Promoter methylation of *SLC6A4* was quantified using pyrosequencing.

Results: We observed that *SLC6A4* expression is upregulated in GTS individuals compared to controls. Although no specific genotype, allele or haplotype was overrepresented in GTS individuals compared to controls, we observed that the L_{AC}/L_{AC} genotype of the 5-HTTLPR/rs25531/rs25532 three-locus haplotype was associated with higher *SLC6A4* mRNA expression levels in GTS individuals, but not in the control group. We observed no association between *SLC6A4* promoter methylation and phenotype, genotype or expression levels.

Conclusions: Our results show that *SLC6A4* expression is increased in GTS individuals, and that this difference is more pronounced in GTS individuals with the L_{AC}/L_{AC} genotype.

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P09.062.C Biallelic frameshift variants in *CYHR1* cause severe global developmental delay

Maria Asif[1,2,3], *Arwa Ishaq A. Khayyat*[1,2], *Salem Alawbathani*[1,2], *Judith Haasters*[4], *Birgit Budde*[1], *Peter Nürnberg*[1], *Matias Wagner*[4,5,6], *Muhammad Sajid Hussain*[1,2,7]

[1]*Cologne Center for Genomics, Faculty of Medicine and University Hospital Cologne, Cologne, Germany,* [2]*Institute of Biochemistry I, Medical Faculty, University of Cologne, Cologne, Germany,* [3]*Center for Molecular Medicine Cologne, University of Cologne, Faculty of Medicine and University Hospital Cologne, Cologne, Germany,* [4]*Department of Pediatric Neurology, Developmental Medicine and Social Pediatrics, Dr. von Hauner's Children's Hospital, University of Munich, Munich, Germany,* [5]*5Institute of Human Genetics, School of Medicine, Technical University of Munich, Munich, Germany,* [6]*Institute for Neurogenomics, Helmholtz Zentrum München, Neuherberg, Germany,* [7]*Center for Molecular Medicine Cologne (CMMC), University of Cologne, Faculty of Medicine and University Hospital Cologne, Cologne, Germany.*

Introduction: The human brain is a highly sophisticated and complex organ, yet to be completely understood. Investigation of neurodevelopmental disorders may unravel protein networks, crucial for brain development.

Material and Methods: We recruited index patients from two families with global developmental delay from Yemen and Germany. To identify the underlying causal variants, we subjected DNA samples of affected members of each family to whole-exome sequencing. Using immunofluorescence, immunoblotting, and pull-down assays coupled with proteomic mass spectrometry (MS), we characterized the protein encoded by the candidate gene and explored the functional consequences of the variants.

Results: In both families, we identified homozygous frameshift variants, c.959_960delTT, p.(Phe320Cysfs*18) and c.1036_1037delCT,

p.(Leu346Glyfs*49) in *CYHR1* (NM_138496.1), a previously uncharacterized gene. In HeLa, MCF7, and human primary fibroblasts, endogenous CYHR1 protein showed predominantly nuclear and only weak cytosolic expression. Transiently expressed wild-type CYHR1 was exclusively observed in the nucleus, however, mutant protein was reduced and found in cytoplasm. Reduction of mutant protein could be recovered by treating the cells with cycloheximide and MG132. In patient derived primary fibroblasts from one case, we observed complete absence of CYHR1. MS analyses identified 110 interacting partners of CYHR1 which were enriched in spliceosome and autophagy related pathways. The autophagy markers LAMP1 and LC3β showed reduced expression in patient derived fibroblasts in line with MS results.

Conclusion: Our findings suggest that loss of CYHR1 causes autosomal recessive neurodevelopmental delay. Hence, CYHR1 is likely to be a novel key factor in human brain development due to impaired autophagy and spliceosome function.

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P09.063.D Glycosylphosphatidylinositol biosynthesis defect due to digenic heterozygous mutations in *PIGT* and *PIGV* genes in a patient with psychomotor and cognitive delay

Gergely Büki[1,2], *Anna Zsigmond*[1], *Agnes Till*[1], *Attila Gyenesei*[3], *Bela Melegh*[1,2], *Kinga Hadzsiev*[1,2], *Judit Bene*[1,2]

[1]*Department of Medical Genetics, Pécs, Hungary,* [2]*Szentagothai Research Center, Pécs, Hungary,* [3]*Genomics and Bioinformatics Research Group, Szentágothai Research Centre, Pécs, Hungary.*

Introduction: Glycosylphosphatidylinositol (GPI) is an anchor for many cell surface proteins. GPI-anchored proteins play crucial roles in many pathways and developmental event, particularly embryogenesis, neurogenesis, immune response and signal transduction. More than 30 genes are involved in the biosynthesis and remodeling of GPI anchor. Mutations in several genes in this pathway have been associated with inherited GPI deficiencies (IGDs) with a wide spectrum of clinical features.

Materials and methods: Whole-genome sequencing was indicated in a male patient with psychomotor and cognitive developmental delay, mild tetraspasticity, rigidity, tremor, reflex anomalies, dystonic hand movements and mild dysmorphic features. Libraries were sequenced on an Illumina NovaSeq 6000 instrument with the help of Illumina TruSeq™ DNA PCR-Free HT Library Prep Kit and 150bp paired-end chemistry. The detected mutations were validated by Sanger sequencing.

Results: Analysis of WGS data revealed a *de novo* heterozygous pathogenic mutation (c.439 C>T) in *PIGV* gene and an unknown, potentially pathogenic heterozygous variant (c.256 C>T) in *PIGT* gene. The patient phenotype shares features related to both gene defect.

Discussion: IGDs are inherited in autosomal recessive and X-linked recessive manner. To our knowledge homozygous or compound heterozygous mutations within the same gene were responsible for the development of IGDs so far. Here we report a male patient carrying digenic heterozygous mutations in *PIGT* and *PIGV* genes which are involved in the same biosynthesis pathway. More and more rare disorders are being recognized with digenic background. NGS technologies (WES and WGS) are effective tools to facilitate the identification of affected patients.

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P09.064.A A novel *GRIN2B* mutation sharing the position but not the phenotypic expression of known pathogenic variant

Nevyana Ivanova, *Kalina Mihova, Kunka Kamenarova, Tsvetina Veleva, Daniela Adjieva-Tzavella, Ivo Kremensky, Vanio Mitev, Ivanka Dimova, Radka Kaneva*

Medical University-Sofia, Sofia, Bulgaria.

Introduction: De novo *GRINB2* mutations are found in a wide range of neurodevelopmental disorders resulting in epileptic encephalopathy and mental retardation with/without epilepsy. Most of them are missense variants clustered in the transmembrane and ligand-binding domains of the N-methyl-D-aspartate (NMDA) receptors showing diverse functional consequences. Here we report a newly identified genetic variant that co-localizes with a previously reported mutation but the related clinical cases exhibit extremely different phenotypic expression.

Materials and Methods: We performed clinical exome sequencing to identify the disease-causing variant in the index patient and the Sanger sequencing method to confirm it and to check its segregation in the family.

Results: Data filtering identified a novel genetic variant c.2090G>A/p.Cys461Tyr in the *GRINB2* gene changes evolutionary conserved amino acid in the ligand-binding domain of the NMDA receptor. Segregation analysis proved that it arises de novo. Mutation was found in a child with only subtle dysmorphic features and mild intellectual disabilities. It affects the same amino acid as a previously reported pathogenic variant (HGMD#CM1314625) replacing cysteine 461 with phenylalanine in a patient with Lennox-Gastaut syndrome.

Conclusions: According to the ACMG criteria the variant c.2090G>A/p.Cys461Tyr in the *GRIN2B* gene is defined as pathogenic. Presence of a much milder clinical phenotype without epilepsy compared to the already reported mutation in the same amino acid causing a severe form of epileptic encephalopathy could be attributed to the different physicochemical properties of the mutant amino acids or imply the influence of modifying genetic factors. Acknowledgements: Funded by Ministry of education and Science D01-285/2019/D01-395/2020.

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P09.065.B Deep phenotyping of biallelic *HACE1* variants

Merope Griffin[1], *Meena Balasubramanian*[2], *Moira Blyth*[3], *Abhijit Dixit*[1], *Peter D. Turnpenny*[4], *Mohnish Suri*[1]

[1]*Clinical Genetics, Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom,* [2]*Sheffield Genetics Service, Sheffield, United Kingdom,* [3]*Yorkshire Regional Genetics Service, Leeds, United Kingdom,* [4]*Clinical Genetics, Royal Devon & Exeter NHS Trust, Exeter, United Kingdom.*

Introduction: Pathogenic and likely pathogenic *HACE1* variants are associated with spastic paraplegia and psychomotor retardation with/without seizures (SPPRS), a rare autosomal recessive, progressive neurodevelopmental disorder characterised by hypotonia, weakness and spasticity of the lower limbs, and seizures. However, the clinical presentation of *HACE1* variants has not been fully characterised. Consequently, we undertook deep phenotyping of 12 patients from 7 families with biallelic *HACE1* variants.

Materials and Methods: Patients with biallelic pathogenic or likely pathogenic *HACE1* variants were identified by trio exome sequencing by the Deciphering Developmental Disorders (DDD) Study or whole genome sequencing by the 100,000 Genomes

Project. A comprehensive phenotyping proforma was completed for all patients by their responsible Clinical Geneticist.

Results: Twelve patients were recruited (7 male/5 female, age range of 2-39 years). Six patients were homozygous for a recurrent nonsense variant (c.805C>T; p.(Arg269Ter)); all these patients were of Pakistani origin. All patients displayed global developmental delay and/or intellectual disability. Ten had seizures, with five having a diagnosis of epilepsy. Notably, six patients had confirmed neurogenic bladder, with one further patient having possible neurogenic bladder that had not been investigated. Four had vesicoureteric reflux. Eye problems (most commonly divergent squint and/or proptosis) were seen in five patients.

Conclusions: Patients with SPPRS variants can present with a distinctive, clinically-recognisable phenotype of developmental delay with early-onset neurogenic bladder, vesicoureteric reflux and ophthalmological problems, potentially aiding early diagnosis of this rare disorder. The pathogenic p.(Arg269Ter) variant appears to be recurrent in the Pakistani population.

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P09.066.C Ataxic neurodegenerative disorders in Sudan: when whole exome sequencing is combined to high consanguinity & old genome

Liena E. O. Elsayed[1,2], *Inaam N. Mohammed*[1], *Ahlam A. A. Hamed*[1], *Maha A. Elseed*[1], *Arwa M. A. Babai*[3], *Elhami A. A. Ahmed*[3], *Doua M. S. AbdelGaleel*[3], *Isra Z. M. Eltazi*[1], *Rayan A. Siddig*[3], *Esraa E. Eltaraife*[3], *Amal S. I. Abd Allah*[1], *Atheer Eltigani*[4], *Nabila M. H. Ali*[1], *Sarah M. Elsadig*[1,5], *Afraa M. M. Musa*[1], *Mahmoud E. Koko*[6], *Ashraf Y. O. Mohamed*[1,7,8], *Mustafa I. Elbashir*[1], *Alexis Brice*[8,9], *Giovanni Stevanin*[8,9,10], *Ammar E. Ahmed*[1], *Muntaser E. Ibrahim*[3]

[1]*Faculty of Medicine, University of Khartoum, Khartoum, Sudan,* [2]*College of Medicine, Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia,* [3]*Department of Molecular Biology, Institute of Endemic Diseases, University of Khartoum, Khartoum, Sudan,* [4]*Department of Medical Biotechnology, Commission for Biotechnology and Genetic Engineering, National Centre for Research, Khartoum, Sudan,* [5]*Department of Neurology, Soba University Hospital, Khartoum, Sudan,* [6]*Department of Neurology & Epileptology, Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany,* [7]*Department of Biochemistry, Faculty of Medicine, National University, Khartoum, Sudan,* [8]*Institut du Cerveau et de la Moelle épinière, INSERM U1127, CNRS UMR7225, Sorbonne Université, UMR_S1127, 75013 Paris, France,* [9]*APHP Pitié-Salpêtrière Hospital, Department of genetics, 75013 Paris, France,* [10]*Ecole Pratique des Hautes Etudes, EPHE, PSL université, 75013 Paris, France.*

Introduction: The highly consanguineous Sudanese population has one of the oldest African genomes with remarkable genetic heterogeneity augmenting the burden of neurogenetic disorders including Hereditary Ataxia (HA). As part of the broader Sudanese neurogenetic project, we explored the genetics underlying the HA in a cohort of 11 families.

Material and Method: Whole exome sequencing (WES) coupled with optimized prioritization approaches was used in 10 families with autosomal recessive HA (ARHA). Phenotype-based candidate genes were tested in the autosomal dominant HA (ADHA) family.

Results: A pathologic CAG repeat expansion in *ATXN7* was found to cause SCA7 with clear maternal anticipation in the ADHA family. In 50% of the ARHA families, novel mutations in known HA genes [*SETX*, *SIL1*, *KIF1C*, and *CLPP*] were found to cause the disease. Potential novel genes pending further validation were identified in the remaining 50%.

Conclusion: The high level of novelty of the results indicates an enormous potential for neurogenetic discoveries in Sudan. The exceptional phenomena observed as the coexistence of multiple genetic disorders in the same of the highly inbred families and intra-familial variation in the phenotypic presentations impose challenges on the analysis of the WES data and necessitate larger-scope studies to fully understand the mechanisms underlying Sudanese neurogenetic disorders. Grants: French Embassy in Sudan, Campus France, University of Khartoum, and the Sudan Ministry of Higher Education for the scholarship (to LE), the Wellcome Trust RDF grant (to LE), the European Union through the H2020 program (SOLVE-RD to GS), and the French ASL-HSP Association (to GS).

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P09.067.D Widening the genetic landscape of syndromic hereditary optic neuropathies

Claudio Fiorini[1,2], *Chiara La Morgia*[2], *Andrea Degiorgi*[3], *Flavia Palombo*[1], *Valerio Carelli*[1,2], *Enrico Baruffini*[3], *Leonardo Caporali*[1]

[1]*IRCCS Institute of Neurological Sciences of Bologna, Bologna, Italy,* [2]*Department of Biomedical and NeuroMotor Sciences, University of Bologna, Bologna, Italy,* [3]*Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parma, Italy.*

Introduction: Hereditary Optic Neuropathies (HON) are a relevant genetic cause of visual impairment, with a reported prevalence of 1/10000 to 1/30000 in Europe. HON typically show a selective loss of Retinal Ganglion Cells and subsequent optic nerve atrophy, moreover, they are often associated with mitochondrial impairment. Most HON patients present mutations in the mitochondrial genome and *OPA1*. Several other genes, mostly related to mitochondrial function, have been identified as rarer causes of non-syndromic or syndromic HON.

Materials and Methods: We performed WES on 75 patients, through an Illumina sequencing platform. Rare variants were then analyzed prioritizing genes related to mitochondrial or neuronal function.

Results: We identified recessive, autosomal, or X-linked pathogenic mutations in 8 cases of HON *plus*. Interestingly, 7 of the identified causative genes (*FDXR, MECR, NDUFAF2, NDUFB11, PDSS1, SLC52A2, WDR45*) were previously associated with severe syndromic phenotypes, also involving optic atrophy, and one in *CACNA1F*, previously associated with night blindness and retinopathy. Instead, our patients were admitted to the diagnostic pipeline as HON, with additional symptoms including neurosensorial hearing loss, neuropathy, developmental delay, ataxia, retinal dystrophy, anemia, ptosis, hypotonia. Those genes are mainly linked to mitochondrial disorders, for which variable expressivity and incomplete penetrance of pathogenic mutations are well established.

Conclusions: Our cases expand the known phenotypic range for rare genes causative for HON *plus*. Mutations in these genes

may occur in cases with optic atrophy "plus", and they must be considered during the diagnostic process. Supported by the Italian Ministry of Health (Grant GR-2016-02361449).

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P09.068.A Somatic mosaicism of the CAG repeat of the *HTT* gene is CAG length- and age-dependent in intermediate (27-35 CAGs) and reduced-penetrance (36-39 CAGs) alleles

Ainara Ruiz de Sabando[1,2], *Marc Ciosi*[3], *Arkaitz Galbete*[2,4], *Darren G. Monckton*[3], *Maria A. Ramos-Arroyo*[1,2]

[1]*Dept. of Medical Genetics, Complejo Hospitalario de Navarra, Pamplona, Spain,* [2]*Navarrabiomed-Complejo Hospitalario de Navarra-Universidad Pública de Navarra, IdiSNA, Pamplona, Spain,* [3]*Institute of Molecular, Cell and Systems Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom,* [4]*Red de Investigación en Servicios de Salud en Enfermedades Crónicas (REDISSEC), Pamplona, Spain.*

Introduction: Huntington Disease (HD) is an autosomal dominant neurodegenerative disorder caused by the expansion of a CAG repeat ($n \geq 36$) in the *HTT* gene. This CAG repeat is somatically unstable in a process that is CAG length-, tissue- and age-specific in HD full-penetrance ($n \geq 40$) alleles. We further investigated CAG somatic expansions in normal ($n \leq 26$), intermediate ($n = 27$-35) and reduced-penetrance ($n = 36$-39) alleles.

Methodology: The *HTT* repeat genotype was determined and the associated somatic mosaicism quantified by Miseq sequencing in blood DNA samples. Samples from 271 individuals carrying *HTT* alleles with 10 to 66 CAGs were analysed. Linear regression was used to study the association of the *HTT* repeat somatic mosaicism with CAG-length and age at sampling.

Results: Somatic mosaicism is allele-length dependent, with a much higher frequency of somatic expansions in larger alleles. We could also observe CAG length-dependent mosaicism in alleles with <40 CAGs ($n = 184$, $b = 0.045$, 95% CI: 0.042-0.048). Moreover, we could show an age-dependent mosaicism in intermediate ($n = 101$) and reduced-penetrance alleles ($n = 37$), with $b = 0.001$ (95% CI: 0.001-0.002) and 0.006 (95% CI: 0.004-0.008), respectively.

Conclusion: We have demonstrated that the CAG repeat in non-HD-causing (CAG < 36) and reduced-penetrance *HTT* alleles is somatically unstable in blood DNA. Because some somatic expansions beyond a particular CAG-length are likely to cause cellular dysfunction, further studies in tissues that drive HD neurodegeneration are warranted, especially for alleles close to the pathologic boundary.

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P09.069.B Patient Survey Evaluating the Experiences of Patients who Participated in at least one Telephone or Virtual Appointment for Pre-Symptomatic Testing for Huntington's disease in the All Wales Medical Genomics Service

Donna Duffin, *Angus Clarke, Karenze Evans, Oliver Murch, Alexandra Murray, Ian Tully, Vani Jain*

Cardiff and Vale University Health Board, Cardiff, United Kingdom.

In response to the on-going global SARS-CoV2 pandemic, the All Wales Medical Genomics Service piloted consultations for pre-symptomatic genetic testing for Huntington's disease via telephone and virtual appointments. Pre-symptomatic testing for incurable neurological conditions has previously only been offered as face-to-face appointments. The patients were contacted prior to their appointments to determine their preferences for appointment type before being taken through the pre-symptomatic testing process. We then surveyed their experiences post-results in order to evaluate our services.

Methods: We conducted structured telephone interviews to gain an insight into their experiences and preferences of the testing processes. This was to determine the impact of having such difficult and emotive discussions while being in their own homes and not in the presence of the Genetic Counsellor or Geneticist. These patients were contacted at least a month after receiving their results.

Results and Conclusions: Although patients had a variety of preferences regarding face-to-face versus telephone or virtual appointments, all appreciated a flexible service where their preferences were considered. It is important to bear in mind there was no one method that suited all patients, and, consequently, we need to remain patient focused in our decision making. Further evaluation needs to be conducted as we continue to deliver our services in these unprecedented times.

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P09.071.D Association between IQ and LSM1 (WHSC1L1) gene polymorphism in Russian students

Andrey V. Marusin[1], *Alexander N. Kornetov*[2], *Maria G. Swarovskaja*[1], *Anna V. Bocharova*[1], *Ksenia V. Vagaitseva*[1], *Ekaterina S. Pavlenyk*[2], *Vadim A. Stepanov*[1]

[1]*The Research Institute for Medical Genetics, National Research Medical Center of Russian Academy of Sciences, Tomsk, Russian Federation,* [2]*Federal State Budgetary Institution of Higher Education "Siberian State Medical University" of the Ministry of Health of Russia, Tomsk, Russian Federation.*

Introduction: Intellectual capability is one of the most socially significant characteristics. Twin studies of adult individuals have found a heritability of IQ between 57% and 73%. Intelligence in the normal range is a polygenic trait, and there are influenced at least 500 genes. However, major intelligence genes, as well as their relationship with neuropsychiatric diseases, have not been identified. Objective of the study is to identify common polymorphic variants of susceptibility to severe behavioral disorders (schizophrenia and Alzheimer's disease) with an intelligence quotient (IQ) total score of young people.

Materials and Methods: The study was carried out on a sample of 135 young people. Using multiplex genotyping by MALDI-TOF method, 29 polymorphic variants in 27 genes were studied. They have previously been known to be associated with Alzheimer's disease or schizophrenia using genome-wide association analysis. The relationship between the studied polymorphic variants and the IQ score was analyzed by using the nonparametric median test.

Results: There were no differences in IQ between men (34) and women (101). Statistically significant associations were found for IQ with r16887244 in the LSM1 gene ($p = 0{,}026$) in case autosomal dominant inheritance (AA vs. GA+GG genotypes). Average IQ values were 113.2, 110.5 and 106.6 for AA, GA and GG genotypes, respectively. Earlier, according to GWAS, this polymorphic variant showed an association with schizophrenia.

Conclusion: The data obtained indicate a common genetic basis for the heritability of mental and neurological disorders with

the intelligence variability. The reported study was funded by RFBR, project number 20-015-00397.

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P09.072.B *PRKN* exon inversion leads to juvenile generalized levodopa-responsive dystonia

Emuna Paz[1], *Hagar Mor-Shaked*[1], *Adily Basal*[1], *Simona Ben-Haim*[2], *Hanna Grobe*[3], *Sami Heymann*[4], *Zvi Israel*[4], *Vardiella Meiner*[1], *Montaser Namnah*[5], *Anat Nitzan*[3], *Ann Saada*[1], *Tomer Tzur*[6], *Ronen Zaidel-Bar*[3], *Tamar Harel*[1], *David Arkadir*[5]

[1]*Department of Genetics, Hadassah Medical Center, Jerusalem, Israel,* [2]*Department of Nuclear Medicine, Hadassah Medical Center, Jerusalem, Israel,* [3]*Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv-Yafo, Israel,* [4]*Department of Neurosurgery, Hadassah Medical Center, Jerusalem, Israel,* [5]*Department of Neurology, Hadassah Medical Center, Jerusalem, Israel,* [6]*Department of Plastic Surgery, Hadassah Medical Center, Jerusalem, Israel.*

Introduction: Biallelic pathogenic variants in *PRKN*, encoding the E3 ubiquitin ligase parkin, lead to autosomal recessive juvenile Parkinson disease [MIM 600116]. Up to 60% of the variants in *PRKN* are structural variants, consistent with its location within FRA6E, one of the most unstable common fragile sites. We describe four siblings afflicted by young onset levodopa-responsive dystonia, in whom genomic testing led to identification of an intragenic inversion disrupting *PRKN*.

Materials and Methods: Following informed consent, exome sequencing and linkage analysis were undertaken on four affected and four unaffected siblings. Whole genome sequencing (WGS) was subsequently pursued on two individuals. Breakpoint junction analysis and analysis of cDNA from patient fibroblasts allowed for characterization of the genomic rearrangement.

Results: All affected individuals shared a homozygous block including *PRKN*. Exome sequencing was not diagnostic. WGS revealed inversion of *PRKN* exon 5, followed by a common 49kb deletion. Breakpoint junction analysis implicated non-homologous end joining as the repair pathway involved. Analysis of cDNA indicated that exon 5 (84bp) was skipped, and was replaced by 93bp of retained intronic sequence, preserving the reading frame yet altering a significant number of residues.

Conclusions: Beyond the common deletions and duplications in *PRKN* associated with juvenile dystonia and Parkinson disease, which may be assessed by read depth analysis of exome data, one must also consider inversions. This study further highlights the complexity of the FRA6E locus and its clinical implications. The authors declare no funding sources for this project.

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P09.074.C A *CLASP1* variant suggests a phenotypic relation with lissencephaly in humans

Zafer Yüksel[1], *Ratna Tripathy*[1], *Norah S. Alsaleh*[2], *Amal Al hashem*[3], *Mojgan Drasdo*[1]

[1]*Center for Human Genetics, Bioscientia Healthcare GmbH, Ingelheim, Germany,* [2]*Division of Medical Genetics and Metabolic Medicine, Department of Pediatrics, Prince Sultan Military Medical City, Riyadh, Saudi Arabia,* [3]*Department of Pediatrics, Prince Sultan Military Medical City, Riyadh, Saudi Arabia.*

Introduction: Lissencephaly is a severe brain developmental disorder, characterized by reduction in brain folding due to underlying cortical layering defects. These aberrations arise during embryonic development from defective neuronal migration, a process in which neurons travel from their place of origin to their final location within the cerebral cortex gray matter. Many of the important roles of microtubule- and actin-associated proteins in regulating the dynamics of the cytoskeleton during neuronal migration have been uncovered through the study of genetic variations that lead to lissencephaly in human and neuronal migration defects in mouse.

Materials and Methods: Whole exome sequencing (WES) analysis was performed in a 10-month-old female and her 13-year-old brother diagnosed with lissencephaly. This analysis rule out all known diagnostic genes described thus far for lissencephaly, following which an approach focusing on disease candidate genes was undertaken along with the informed consent of the family.

Results: WES revealed a homozygous missense variant in CLASP1 (OMIM: 605852) [NM_015282.2:c.4442G>A p.(Arg1481His)] in the affected siblings. Both parents are heterozygous carriers.

Conclusion: Cytoplasmic linker-associated protein 1 (CLASP1) belongs to a group of proteins known as CLASPs which are non-motor microtubule-associated proteins that interact with CLIPs (Cap-Gly Domain-containing linker protein), a member of the microtubule plus-end tracking protein family. In this work, we propose the importance of CLASP1 as a diagnostic gene, wherein variants in CLASP1 lead to brain structural disorders such as lissencephaly in humans.

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P09.075.D A case of posterior lissencephaly due to a variation in *CEP85L* gene: case report and refining of the phenotypic spectrum

Gianluca Contrò[1], *Alessia Micalizzi*[2], *Sara Giangiobbe*[1,3], *Simonetta Rosato*[1], *Marzia Pollazzon*[1], *Stefano Giuseppe Caraffi*[1], *Gabriele Trimarchi*[1], *Susanna Rizzi*[4], *Francesca Clementina Radio*[5], *Nives Melli*[6], *Carlo Fusco*[7], *Giancarlo Gargano*[6], *Marco Tartaglia*[5], *Antonio Novelli*[5], *Livia Garavelli*[1]

[1]*Medical Genetics Unit, Azienda USL, IRCCS, Arcispedale Santa Maria Nuova, Reggio Emilia, Italy,* [2]*Laboratory of Medical Genetics, Bambino Gesù Children's Hospital, Rome, Italy,* [3]*Clinical Genomics, Medical Genetics Service, San Raffaele Hospital, Milan, Italy,* [4]*Child Neurology and Psychiatry Unit, Reggio Emilia, Italy,* [5]*Genetics and Rare Diseases Research Division, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy,* [6]*Neonatal Intensive Care Unit (NICU), Obstetric and Pediatric Department, Azienda USL-IRCCS di Reggio Emilia, Reggio Emilia, Italy,* [7]*Child Neurology and Psychiatry Unit, Azienda USL- IRCCS di Reggio Emilia, Reggio Emilia, Italy.*

Lissencephaly describes a group of clinical conditions characterized by the absence of normal cerebral convolutions and abnormalities of cortical development. To date, almost 20 genes have been identified as causative of this condition. Variants in *CEP85L*, encoding a protein involved in the regulation of neuronal migration, have been described as causative of this condition with a prevalent involvement of the posterior cerebral cortex and an autosomal dominant patter of inheritance. Here we describe a 5-month-old boy with delayed

psychomotor development and mild phenotypic features (such as bitemporal narrowing, slightly protruding ears with up-lifted lobes, posterior plagiocephaly). In his clinical history, bordeline ventriculomegaly was observed during the antenatal ultrasound scan. EEG showed a discontinuous pattern with interemispheric asymmetry and a cortical electrogenesis disorder in the absence of epileptiform abnormalities. MRI identified lissencephaly type 1, prevalently in the temporo-occipito-parietal regions of both sides with "double-cortex" (Dobyns' 1-2 degree) periventricular band alterations. Furthermore, a patent foramen ovale was detected. Among the genetic tests no alterations was identified by array-CGH and NGS-panel for cortical malformations, but whole-exome sequencing revealed the presence of a previously unreported *de novo* pathogenic variant in the *CEP85L* gene (c.232+1delG, NM_206921). To date, only 13 patients showing lissencephaly with prevalent posterior involvement, variable cognitive deficits and epilepsy have been reported as carriers of pathogenic *CEP85L* variants. The present findings document that *CEP85L* mutations are not necessarily associated with severe phenotypes and relevant MRI alterations, documenting the importance of including *CEP85L* among the genes involved in the pathogenesis of lissencephaly.

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P09.076.A The clinical and neuroradiological spectrum of variants in the GAR domain of MACF1

Jordy Dekker[1], *Rachel Schot*[1], *Kimberly A. Aldinger*[2], *David B. Everman*[3], *Jennifer A. Sullivan*[4], *Vandana Shashi*[4], *Maha S. Zaki*[5], *Joseph G. Gleeson*[6], *Antonio Vitobello*[7,8], *Anne-Sophie Denommé-Pichon*[7,8], *Anne-Laure Mosca-Boidron*[7,8], *Sophie Nambot*[7], *Laurence Perrin*[9], *Stéphane Auvin*[9,10], *Sarah E. McKeown*[11,12], *Mark P. Fitzgerald*[11,12], *Ingo Helbig*[11,12], *Felice D'Arco*[13], *Richard J. Leventer*[14], *Dan Doherty*[2,15], *William B. Dobyns*[15,16], *Grazia M. S. Mancini*[1], *Kevin C. Slep*[17]

[1]*Erasmus MC, Rotterdam, Netherlands,* [2]*Seattle Children's Research Institute, Seattle, WA, USA,* [3]*Greenwood Genetic Center, Greenwood, SC, USA,* [4]*Duke University School of Medicine, Durham, NC, USA,* [5]*Human Genetics and Genome Research Division, Cairo, Egypt,* [6]*Howard Hughes Medical Institute, San Diego, CA, USA,* [7]*Université de Bourgogne, Dijon, France,* [8]*Centre Hospitalier Universitaire de Dijon, Dijon, France,* [9]*Hôpital Robert Debré, Paris, France,* [10]*Université de Paris, Paris, France,* [11]*The Children's Hospital of Philadelphia and the Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA,* [12]*Children's Hospital of Philadelphia, Philadelphia, PA, USA,* [13]*Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom,* [14]*Royal Children's Hospital, Melbourne, Australia,* [15]*University of Washington, Seattle, WA, USA,* [16]*University of Minnesota, Minneapolis, MN, USA,* [17]*University of North Carolina, Chapel Hill, NC, USA.*

Microtubule-actin cross-linking factor 1 (MACF1) is a member of the spectraplakin protein family, that cross-link different components of the cytoskeleton. The growth arrest specific 2 (Gas2)-related (GAR) domain of MACF1 interacts with microtubules and dominant variants affecting the GAR domain result in a brain malformation involving a predominant posterior lissencephaly and reduced or absent pontine crossing fibers resulting in a W-shaped hypoplastic brainstem. Here we describe six patients with a *de novo* variant in the GAR domain of *MACF1*, of which five have not been reported before. We also identified a variant in the most N-terminal of the four zinc-binding residues (NM_012090.5:c.15524G>A p.(Cys5175Tyr)), where previously no other variants have been reported. All patients with a zinc-binding residue variant show on MRI a severe narrowing of the pons, cerebellar vermis hypoplasia and a variable lissencephaly severity. Patients present with global developmental delay, having impaired motor development and speech development ranging from no speech to using single words. Most patients develop epilepsy during their first year after birth. Furthermore, we identified one patient with a variant located between the two zinc-binding residue pairs (c.15575G>C p.(Arg5192Pro)), likely to affect the β-sheet structure of the GAR domain, whose MRI reveals only mild brainstem and cerebellar hypoplasia with cortical dysgyria, but no lissencephaly. These results show that variants in the zinc-binding residues of the MACF1 GAR domain result in a typical cortical malformation, where variants in the surrounding residues may be associated with a different phenotype.

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P09.077.B Genetic contributions to psychopathological symptom dimensions in a transdiagnostic cohort of patients with major depressive disorder, bipolar disorder, schizophrenia, and schizoaffective disorder

Friederike S. David[1], *Frederike Stein*[2,3], *Till F. M. Andlauer*[4], *Tilo Kircher*[2,3], *Udo Dannlowski*[5], *Sergi Papiol*[6,7], *Urs Heilbronner*[7], *Peter Falkai*[6], *Thomas G. Schulze*[7], *Marcella Rietschel*[8], *Markus M. Nöthen*[1], *Axel Krug*[9,2], *Andreas J. Forstner*[1,10,11]

[1]*Institute of Human Genetics, University of Bonn, School of Medicine & University Hospital Bonn, Bonn, Germany,* [2]*Department of Psychiatry and Psychotherapy, University of Marburg, Marburg, Germany,* [3]*Center for Mind, Brain and Behavior, University of Marburg, Marburg, Germany,* [4]*Department of Neurology, Klinikum rechts der Isar, School of Medicine, Technical University of Munich, Munich, Germany,* [5]*Department of Psychiatry and Psychotherapy, University of Münster, Münster, Germany,* [6]*Department of Psychiatry and Psychotherapy, University Hospital, LMU Munich, Munich, Germany,* [7]*Institute of Psychiatric Phenomics and Genomics (IPPG), University Hospital, LMU Munich, Munich, Germany,* [8]*Department of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany,* [9]*Department of Psychiatry und Psychotherapy, University of Bonn, Bonn, Germany,* [10]*Institute of Neuroscience and Medicine (INM-1), Research Center Jülich, Jülich, Germany,* [11]*Centre for Human Genetics, University of Marburg, Marburg, Germany.*

Introduction: Major depressive disorder (MDD), bipolar disorder (BD), schizophrenia (SCZ), and schizoaffective disorder (SZA) are a group of psychiatric disorders with a considerable amount of phenotypic and genetic overlap. Recently, Stein et al. (2020) have developed a 5-factor model that captures transdiagnostic symptom dimensions in these disorders. Here we investigate underlying genetic factors for the five symptom dimensions (depression, negative syndrome, positive formal thought disorder, paranoid-hallucinatory syndrome, and increased appetite).

Methods: Our transdiagnostic sample (n = 1042) of individuals diagnosed with MDD, BD, SCZ, or SZA was recruited from the German FOR2107 cohort. In this sample we performed genome-wide association studies (GWAS) for each of the five symptom dimensions using the linear regression approach in PLINK. For the most significant finding, we conducted a replication analysis in an

independent sample (n = 875) of the German PsyCourse cohort based on an approximate phenotype measure.

Results: The discovery GWAS revealed between 5 and 16 suggestively associated loci ($P < 1x10^{-5}$) for each symptom dimension. For the dimension of positive formal thought disorder, we identified one genome-wide significant association ($P < 5x10^{-8}$) on chromosome 10 in the FOR2107 discovery sample. However, this association could not be replicated in the PsyCourse sample.

Conclusions: While our findings suggest that genetic factors contribute to transdiagnostic symptom dimensions in MDD, BD, SCZ, and SZA and thus indicate shared etiological factors, no replicable genome-wide associations could be identified at the given sample sizes. Additional studies with larger sample sizes are required to further elucidate the genetic contributions to transdiagnostic symptom dimensions.

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P09.079.D DNA methylation pattern of gene promoters of *MB-COMT, DRD2,* and *NR3C1* in Turkish patients diagnosed with schizophrenia

Hasan Mervan Aytac[1], *Yasemin Oyaci*[2], *Mustafa Pehlivan*[3], *Sacide Pehlivan*[2]

[1]*Psychiatry Unit, Malazgirt State Hospital, Mus, Turkey,* [2]*Department of Medical Biology, Istanbul Faculty of Medicine, Istanbul University, Turkey, Istanbul, Turkey,* [3]*Department of Hematology, Gaziantep University, Faculty of Medicine, Gaziantep, Turkey, Gaziantep, Turkey.*

Objective: We aim to evaluate the methylation status of *MB-COMT* promotor, *DRD2*, and *NR3C1* gene in patients with schizophrenia (SCZ) by comparing healthy controls.

Methods: A sample of 110 patients with SCZ and 100 age- and sex-matched healthy volunteers was included in the study. The interview was started by filling out data forms that included sociodemographic and clinical information. SCID-I was used to confirming the diagnosis according to DSM-IV-TR criteria. Then the patients were evaluated with the PANSS in terms of symptom severity. Methylation-specific polymerase chain reaction (MSP-PCR) was used to determine the methylation status of *MB-COMT* promotor, *DRD2*, and *NR3C1* gene from DNA material.

Results: When we compared the percentages of *MB-COMT* promotor, *DRD2*, and *NR3C1* gene methylation status in SCZ patients with the healthy control group, the percentages of *MB-COMT* promotor (OR: 0.466; 95% CI: 0.268-0.809; p = .006), *DRD2* (OR: 0.439; 95% CI: 0.375-0.514; p < .001), and *NR3C1* (OR: 0.003; 95% CI: 0.001-0.011; p < .001) gene methylation status of SCZ was found to be significantly different from the control group. Whereas unmethylation of *MB-COMT* promotor and *NR3C1* genes were associated with SCZ, the partial methylation of the *NR3C1* gene was related to the SCZ.

Conclusion: The *MB-COMT* promotor, *DRD2*, and *NR3C1* gene methylation status may be associated with the SCZ in the Turkish population.

Comparison of frequencies of MB-COMT, DRD2 and NR3C1 methylation between SCZ patients with control

Methylation	Genotype	Schizophrenia	Healthy Control	OR	95% CI	p*
		n = 110 (%)	n = 100 (%)			
COMT	**Unmethylation**	68 (61.8)	43 (43.0)	0.466	0.268-0.809	**.006***
	Partial Methylation	42 (38.2)	57 (57.0)	0.466	0.268-0.809	
DRD2	**Unmethylation**	0 (0.0)	14 (14)	0.439	0.375-0.514	**.000***
	Partial Methylation	110 (100.0)	86 (86)	0.439	0.375-0.514	
NR3C1	**Unmethylation**	107 (97.3)	9 (9)	0.003	0.001-0.011	**.000***
	Partial Methylation	3 (2.7)	91 (91)	0.003	0.001-0.011	
Abbreviations	*, Pearson chi-square	OR, odds ratio	CI, confidence interval			

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P09.080.A Chromosomal microarray analysis in 97 pediatric cases of microcephaly

Ana-Maria Meašić[1], *Adriana Bobinec*[1], *Leona Morožin Pohovski*[2], *Ivona Sansović*[1], *Mijana Kero*[1], *Ljubica Boban*[1], *Ingeborg Barišić*[1]

[1]*Department of Medical Genetics and Reproductive Health, Children's Hospital Zagreb, Scientific Centre of Excellence for Reproductive and Regenerative Medicine (CERRM), University of Zagreb School of Medicine, Zagreb, Croatia,* [2]*Department of Medical Genetics and Reproductive Health, Children's Hospital Zagreb, University of Zagreb School of Medicine, Zagreb, Croatia.*

Introduction: Microcephaly is defined as an occipitofrontal head circumference (OFC) more than 2 standard deviations (SD) below the mean for sex and age. It is associated with a reduction in brain volume and often developmental/intellectual disabilities. The pathogenesis is heterogeneous, ranging from genetic to environmental factors. Anomalies may exclusively affect cerebral development (non-syndromic) or may include extracranial malformations and/or facial dysmorphism (syndromic).

Materials and Methods: A retrospective analysis was performed on the results of whole-genome chromosomal microarray analysis (CMA) of 97 children with microcephaly evaluated by clinical geneticists in the period from 2015-2020.

Results: Twenty-six pathogenic copy number variants (CNVs) were detected in 23 patients presenting syndromic microcephaly with diagnostic yield of 23.71%. Slightly higher diagnostic yield was observed associated with severe (OFC more than 3 SD below the mean), 26.42%, than mild microcephaly, 20.45%. Certain phenotypes predicted for the presence of pathogenic CNV, especially combined with severe microcephaly, including brain anomalies (odds ratio [OR] 2.03 [0.78-5.28]), cardiovascular anomalies (OR 4.96 [1.47-16.78]), skeletal anomalies (OR 2.75 [1.05-7.20]) and short stature (OR 2.29 [0.81-6.45]).

Conclusion: The results support the use of CMA as first-tier test for syndromic microcephaly, especially for severe microcephaly presented with skeletal and/or cardiovascular anomalies. Other comorbidities, such as other brain anomalies, neurological abnormalities and short stature, may also increase the diagnostic yield. Acknowledgement: This study was supported by CERRM, Republic of Croatia, and by the EU through ERDF, under grant agreement No. KK.01.1.1.01.0008, project„Reproductive and Regenerative Medicine - Exploring New Platforms and Potentials".

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P09.081.B A rare case of microlissencephaly associated with *KATNB1* gene variants

Ana Grangeia[1,2], *Carina Couto Reis*[3], *Miguel Leão*[1]

[1]*Medical Genetics Service, Centro Hospitalar e Universitário São João (CHUSJ), Porto, Portugal,* [2]*Department of Genetics, Faculty of Medicine (FMUP), University of Porto, Porto, Portugal,* [3]*Neurorradiology Service, Centro Hospitalar e Universitário São João (CHUSJ), Porto, Portugal.*

Introduction: In 2014, pathogenic variants in *KATNB1* gene, that encodes the regulatory p80 subunit of Katanin, a microtubule-severing enzyme, have been shown to cause microcephaly with brain malformation, particularly a simplified gyral pattern. Since then, only 9 families were described world-wide, 8 of them consanguineous, harbouring *KATNB1* variants in homozygosity. Case Report: We present a 14-years-old Portuguese girl born of non-consanguineous healthy parents, who was referred for genetic evaluation due to congenital microcephaly. Physical exam at birth and throughout life revealed microcephaly, strabismus, nystagmus and moderate psychomotor development delay. Brain MRI demonstrated frontal bilateral and symmetrical lissencephaly and pachygyria, parieto-occipital polymicrogyria, subcortical heterotopia and hypoplasia of the cerebellar vermis. Chromosomal microarray and a new generation sequencing panel targeting key genes involved in neuronal migration disorders revealed no causative variants. Clinical exome sequencing revealed two heterozygote variants, c.1567-23_156722del and c.1703_1718+25del in the *KATNB1* gene. Both variants occurred with very low frequencies in Genome Agrregation Database and were not reported in the literature or disease ClinVar database. In silico tools predicted that c.1567-23_1567-22del and c.1703_1718+25del variants alter splicing by disrupting the branch point in the intron 13 and by deleting the acceptor splice-site of intron 18 of the *KATNB1* gene, respectively. Segregation analysis and mRNA studies for functional evaluation are in course.

Conclusion: Here we report the second patient with congenital microcephaly caused by two heterozygotes variants in *KATB1* gene. This study supports the fundamental role of KATNB1 in human cerebral cortical development and pathology and extend the genotype of KATNB1-associated microlissencephaly.

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P09.082.C Analysis of the most common nuclear genome encoded mitochondrial gene in Hungarian patients with adult-onset mitochondrial disorders

Fruzsina Szabo[1], *Zoltan Grosz*[1], *Andras Gezsi*[2], *Anna Suveges*[1], *Idris J Jimoh*[1], *Helga Zeke*[1], *Aniko Gal*[1], *Mária Judit Molnár*[1]

[1]*SOTE, Institute of Genomic Medicine and Rare Disorders, Budapest, Hungary,* [2]*BME, Faculty of Electrical Engineering and Informatics (VIK) Department of Measurement and Information Systems, Budapest, Hungary.*

Introduction: Mitochondrial disease is one of the most common metabolic diseases with a minimum prevalence of greater than 1 in 5000 in adults. The disease may affect multiple organs and could be inherited both dominant, recessive, X linked way or maternally depending. Beside mtDNA nuclear genes are responsible for the mitochondrial function. In this study the genetic background of adult onset mitochondrial disorders were investigated in Hungarian patients.

Materials and Methods: In our study, 75 patients (28 men and 47 women) (mean age: 49.9 ± 13.5) with multi-systemic phenotype were tested. The inclusion criteria was a muscle biopsy and/or lactate stress test indicating mitochondrial disease. Targeted panel NGS sequencing was performed investigating 167 nuclear genes **Results:** *In* more than 50% of the cohort the main presenting symptom was myopathy. Progressive ophthalamoplegia externa, ataxia, psychiatric symptoms, and diabetes mellitus was common as well. Pathogenic or likely pathogenic mutations were found in 6 cases in heterozygous form (3 *SPG7*, 1 *MSTO1*, 1 *NDUFV1*, 1 POLG2).

Conclusion: In our cohort heterozygous pathogenic or likely pathogenic alterations have been found in 8%. The pathomechanisms of adult-onset disease form is poorly studied, so we do not have enough information on the exact intracellular effect of single heterozygous mutations, therefore these cases further functional test might be needed.This study was supported by KTIA_NAP_2017-1.2.1-NKP-2017-00002; NKIH_132812 gratnts and FIKP program. The Institute is the part of ERN RND and NMD.

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P09.084.A Mutations, genes and phenotypes related to movement disorders: a never-ending list

Dolores Martínez-Rubio[1,2], *Paula Sancho*[1,2], *Isabel Hinarejos*[1,2], *Itxazo Martí*[3], *S Jesús-Maestre*[4], *Raquel Baviera*[2,5], *I Sastre*[2,5], *Irene Martínez*[2,5], *Marta Correa-Vela*[6], *Cristina Tello*[1], *Amparo Andrés-Bordería*[1,2], *Ana Sánchez-Monteagudo*[1,2], *Alejandra Darling*[7], *A Duat*[8], *P Janeiro*[9], *E Moreno*[10], *M J. Martínez-González*[11], *A Ruiz*[12], *Vincenzo Lupo*[1,2], *P Mir*[4], *Belén Pérez-Dueñas*[13], *Sergio Aguilera*[14], *Carmen Espinós*[1,2]

[1]*Unit of Rare Neurodegenerative Diseases, Centro de Investigación Príncipe Felipe, Valencia, Spain,* [2]*Rare Diseases Joint Unit, CIPF-IIS La Fe, Valencia, Spain,* [3]*Department of Pediatrics, Hospital U. Donostia, San Sebastián, Spain,* [4]*Unit of Movement Disorders, Instituto de Biomedicina de Sevilla, Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Seville, Spain,* [5]*Department of Neurology, Hospital Universitari i Politècnic La Fe, Valencia, Spain,* [6]*Department of Pediatrics, Hospital Universitario Virgen del Rocío, Seville, Spain,* [7]*Department of Pediatrics, Hospital Sant Joan de Déu, Barcelona, Spain,* [8]*Department of Pediatrics, Hospital Infantil Universitario Niño Jesús, Madrid, Spain,* [9]*Department of Pediatrics, Hospital de Santa Maria, Lisbon, Portugal,* [10]*Department of Pediatrics, Hospital Regional, Málaga, Spain,* [11]*Department of Pediatrics, Hospital Universitario de Cruces, Barakaldo, Spain,* [12]*Department of Pediatrics, Hospital Universitari Son Espases, Palma, Spain,* [13]*Department of Pediatrics, Hospital Universitari Vall d'Hebron, Barcelona, Spain,* [14]*Department of Pediatrics, Complejo Hospitalario de Navarra, Pamplona, Spain.*

Introduction: Movement Disorders (MDs) comprise heterogeneous neurological syndromes that present dysfunction in the basal ganglia and/or connected structures. Patients may present with ataxia, parkinsonism, dystonia, chorea, spasticity, myoclonus, tremor, and others. Hundreds of genes are associated with MDs and genetic diagnosis in clinical practice may end up being a cumbersome odyssey.

Material and Methods: We investigated a clinical series with 54 patients suffering from MDs using a custom panel based on SureSelectQXT technology (Agilent Technologies), which comprises 498 genes. To compare both approaches, 10 additional patients were studied by whole exome sequencing (WES), using the whole exome family plus test (Blueprint Genetics) or Human Clinical Exome Capture & Mitochondrial DNA (Nimblegen).

Results: Using the panel MovDisord-498, 20 patients achieved a genetic diagnosis. Nine patients were further investigated by WES, and 3 of them were solved. On the other hand, in 6 out of the 10 patients only studied by WES, the causative changes were identified. All in all, we detected 34 mutations, 19 of them being novel, in 21 different genes in 26 out of 64 probands.

Conclusions: The genetic bases of MDs show an incredible heterogeneity. The obtained diagnosis rate success (45.3%) is a pretty nice rate, although insufficient. We need to unravel the molecular bases underlying these Mendelian disorders whose molecular causes escape to the prevailing techniques because there are a notable proportion of patients who remain without diagnosis. Grants: ISCIII co-funded with ERDF funds [PI18/00147], the Fundació La Marató TV3 [20143130-31], Generalitat Valenciana [PROMETEO/2018/135].

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P09.085.B Quantitative dissection of multilocus pathogenic variation

*Isabella Herman[1], **Angad Jolly**[1], Haowei Du[1], Moez Dawood[1], Ghada M. H. Abdel-Salam[2], Dana Marafi[1], Tadahiro Mitani[1], Daniel G. Calame[1], Zeynep Coban-Akdemir[3], Jawid M. Fatih[1], Ibrahim Hegazy[2], Shalini N. Jhangiani[1], Richard A. Gibbs[1], Davut Pehlivan[1], Jennifer E. Posey[1], James R. Lupski[1]*

[1]Baylor College of Medicine, Houston, TX, USA, [2]National Research Centre, Cairo, Egypt, [3]The University of Texas Health Science Center, Houston, TX, USA.

Objective: Genomic sequencing and clinical genomics have demonstrated substantial subsets of atypical and/or severe disease presentations result from multilocus pathogenic variation (MPV) causing blended phenotypes. Using the Human Phenotype Ontology (HPO), we quantitatively dissected the blended phenotype of an infant with severe neurodevelopmental disorder, brain malformation, dysmorphism, and hypotonia.

Methods: Family-based exome sequencing (ES) with rare variant analysis was completed. HPO analysis with semantic similarity was implemented to determine phenotypic contribution of each implicated gene.

Results: ES revealed deleterious variants in *CAPN3* (c.259C>G:p.L87V), *MUSK* (c.1781C>T:p.A594V), *NAV2* (c.1996G>A:p.G666R), and *ZC4H2* (c.595A>C:p.N199H). *CAPN3, MUSK*, and *ZC4H2* are established disease genes linked to limb-girdle muscular dystrophy (OMIM# 253600), congenital myasthenia (OMIM# 616325), and Wieacker-Wolff syndrome (OMIM# 314580), respectively. *NAV2* is a retinoic-acid responsive novel gene candidate with biological roles in neurite outgrowth and cerebellar dysgenesis in mouse models. Using semantic similarity, we show quantitatively that no gene individually explains the proband phenotype but rather the totality of the clinically observed disease is most parsimoniously explained by disease-contributing effects of all four genes. These data reveal that the combination of variants results in a blended phenotype with each gene affecting a different part of the nervous system and nervous system-muscle connection.Interpretation: In patients with MPV and complex blended phenotypes resulting from multiple molecular diagnoses, HPO analysis allows for dissection of phenotypic contribution of both established disease genes and novel gene candidates not yet proven to cause human disease with marked implications for prognosis, treatment, and family counseling for the most complex genetic patients.

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P09.086.C Multiple sclerosis associated HLA variants affect the immunological T lymphocytes repertoire

***Melissa Sorosina**[1], Silvia Santoro[1], Laura Ferrè[1,2], Elisabetta Mascia[1], Ferdinando Clarelli[1], Antonino Giordano[1,2], Miryam Cannizzaro[1,2], Massimo Filippi[2,3,4], Federica Esposito[1,2]*

[1]Laboratory of Neurological complex disorders, Division of Neurosciences, IRCCS San Raffaele Scientific Institute, Milan, Italy, [2]Neurology, Neurorehabilitation and Neurophysiology Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy, [3]Neuroimaging Research Unit, Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Milan, Italy, [4]Vita-Salute San Raffaele University, Milan, Italy.

Introduction: genetic predisposition to multiple sclerosis (MS) includes >200 genetic loci, with the major histocompatibility complex (MHC) region accounting for 32 independent associations. We aim to investigate the impact of MHC MS-risk alleles on T-lymphocytes repertoire in MS.

Methods: 183 untreated relapsing-remitting MS subjects have been studied. Class I and II HLA alleles were inferred from whole-genome genotyping data using SNP2HLA and Beagle_v3.3 tools. T-cell receptor (TCR) CDR3 sequences were obtained from whole blood DNA according to the ImmunoSEQ hsTCRB kit (Adaptive Biotechnologies®). The weighted HLA-risk score (wHRS) was calculated for each individual. The inverse of the Simpson's Index (INV.S) was calculated as representative of immune repertoire diversity. Statistical analyses were performed within R environment and plink v.1.9.

Results: after quality controls, the final set was composed by 144 individuals and 30 MS-risk MHC loci. Four loci showed association with INV.S: HLA DRB1*15:01 ($P = 0.014$), rs11751659 ($P = 0.02$), rs9271366 ($P = 0.003$), SNP_DRB1_32660116_A ($P = 0.036$). A mild association was found between INV.S and wHRS ($P = 0.049$), with individuals with a higher wHRS showing a lower diversity. Additionally, individuals carrying the risk alleles showed a different percentage of clonotypes occupying the 10% of the repertoire.

Conclusions: MS-risk MHC loci appear to influence TCR repertoire in MS patients, with the risk alleles reducing the diversity and inducing an expansion of specific clonotypes. Analyses are ongoing to better define the amplified clonotypes and their role.

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P09.087.D Differentially expressed genes and their miRNA regulators in Multiple Sclerosis

Nagehan Ersoy Tunali, *Birgül Çolak*

Istanbul Medeniyet University, Istanbul, Turkey.

Multiple Sclerosis (MS) is an immune-mediated disorder, resulting in demyelination of the neurons. Gene expression changes in T and B cells are considered to be the main initiator of disease pathology. In this work, we aimed to investigate the differentially expressed genes (DEGs) in T cells in MS using bionformatic tools and to identify the miRNAs regulating these genes. For this purpose, we used GSE43591 dataset, which contains microarray profiling data obtained from 10 RRMS and 10 healthy individuals. We first determined differentially expressed genes (DEGs), then we applied functional enrichment analysis using DAVID. Pathway enrichment is accomplished by KEGG pathway analysis. Protein-protein interaction (PPI) network was constructed using STRING database, then transferred to Cytoscape to screen for the hub proteins. Finally, we screened for the targeting miRNAs for each hub-protein-expressing genes. 582 nodes and 2102 edges were mapped in the PPI network of identified DEGs, including 388 up-regulated and 1318 down-regulated genes. The 10 hub proteins include RBX1, UBA52, SKP1, FBXW11, UBE2B, CDC34, UBE2R2, UBE3A, HERC5 and FBXL14. The KEGG pathway analysis showed that the up-regulated genes were significantly enriched in ubiquitin mediated proteolysis, RNA degradation and NF-kappa B signalling, while down-regulated genes were significantly enriched in B and T cell receptor signalling, neurotrophin signalling, NK cell mediated cytotoxicity and apoptosis. The miRNAs targeting the genes encoding 10 hub proteins were miR514b-3p, miR495-3p, miR3913-5p, miR4420, miR4789-5p, miR4500, miR4725-3p, miRNA-374b-5p, miR-196a-1-3p, miR5011-5p. We conclude that DEGs in Tcells are mostly involved in ubiquitination and signalling in the immune system.

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P09.088.A A replication study of genetic variants associated with multiple sclerosis risk in the Kuwaiti population

Rabeah A. Altemaimi[1], *Khadijah Ateyah*[1], *Mohammad Dashti*[2], *Raed Alroughani*[3]

[1]*Kuwait University, Jabriya, Kuwait,* [2]*Dasman Dlabetes Institute, Sharq, Kuwait,* [3]*Amiri Hospital, Kuwait City, Kuwait.*

Introduction: Multiple Sclerosis (MS) is a chronic neurodegenerative disorder resulting from an autoimmune reaction against myelin. Many genetic variants have been reported to associate with MS risk however their association is inconsistent across populations. Here we investigated the association of consistently reported genetic MS risk variants in Kuwaiti MS patients.

Materials and Methods: Fifty-six healthy Kuwaitis and 113 Kuwaiti MS patients were exome sequenced on Illumina's HiSeq2000, and 404 healthy Arab control exomes from public databases were used. Bioinformatic analysis was used to mine for 94 MS related risk variants with ≥ 2 reports confirming MS risk association. Replication analysis was done on 170 MS patients and 311 healthy Kuwaitis using Taqman genotyping assays.

Results: Of the 94 reported MS risk variants four showed MS risk association in the exome analysis (*EVI5* rs11808092 $p = 0.0002$; *TNFRSF1A* rs1800693 $p = 0.00003$; *MTHFR* rs1801131 $p = 0.038$; and *CD58* rs1414273 $p = 0.00007$). Replication analysis in only Kuwaiti cohorts confirmed *EVI5* rs11808092A, *TNFRSF1A* rs1800693C, and *MTHFR* rs1801131G as MS risk factors in the Kuwaiti population (OR: 1.6, 95%CI: 1.19 - 2.16, $p = 0.002$; OR: 1.36, 95%CI: 1.04 - 1.78, $p = 0.025$; and OR: 1.79, 95%CI: 1.3 - 2.36, $p = 0.001$; respectively). *CD58* rs1414273 did not sustain risk association ($p = 0.37$).

Conclusions: Variants in *EVI5*, *TNFRSF1A* and *MTHFR* are MS risk factors in the Kuwaiti population. Further investigations into their roles in MS pathogenesis and progression are merited.This work was funded by KFAS grant 2012-1302-02.

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P09.090.C Neuronal Ceroid Lipofuscinoses 6 type in Yakutia

Polina Golikova[1], *Aitalina Sukhomyasova*[1,2], *Elizaveta Gurinova*[2], *Irina Nikolaeva*[2], *Diana Petukhova*[1], *Svetlana Stepanova*[2], *Roza Ivanova*[1,2], *Tatyana Grigorieva*[1,2], *Tatyana Nikolaeva*[3], *Nadezda Maksimova*[1]

[1]*Research Laboratory "Molecular Medicine and Human Genetics", Medical Institute, M.K. Ammosov North-Eastern Federal University, Yakutsk, Russian Federation,* [2]*Medical Genetic Center, Republican Hospital №1 - "National Medical Center", Yakutsk, Russian Federation,* [3]*Medical Institute, M.K. Ammosov North-Eastern Federal University, Yakutsk, Russian Federation.*

Introduction: the neuronal ceroid lipofuscinoses (NCLs) are a group of lysosomal storage disorders. NCL is the most common childhood neurodegenerative disease with a prevalence of 1:1000000 to 1:14000 worldwide.

Methods: in the Medical Genetic Center of National Medical Center (Yakutsk) a few families with a clinical diagnosis of leukodystrophy were observed. All observed cases were Yakut nationality. In order to search for a molecular genetic cause of disease in patients we carried out the exome sequencing using the TruSight Inherited Disease panel (Illumina, USA). The results were validated by Sanger direct sequencing. Experiments were performed using equipment of Center for collective use of the North-Eastern Federal University.

Results: we identified a homozygous frameshift variant c.396dupT (p.Val133CysfsTer18) in 4th exon of *CLN6* gene in all patients. Their parents were heterozygous carriers of the mutation. Totally we revealed 22 patients from 18 unrelated Yakut families with a diagnosis of type 6 neuronal ceroid lipofuscinosis. The main clinical symptoms of disease were early onset of the disease at the age of 3-4 years, impaired coordination, frequent falls, regression of psychomotor development, seizures, subatrophy of the optic nerves. The total prevalence of NCL6 in Yakutia is 2.3 per 100000 populations.

Conclusion: All examined patients with NCL6 had one major mutation in the *CLN6* gene. The results obtained can be useful for molecular genetic diagnostics and consultations. Grant: The study was supported by the Ministry Education and Science of Russian Federation (Project No. FSRG-2020-0014 "Genomics of Arctic: epidemiology, hereditary and pathology")

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P09.091.D The portray of the Italian cohort of patients with variants in *POGZ:* new care opportunities from a deep genotyping and phenotyping

Agnese Feresin[1], *Beatrice Spedicati*[1], *Giulia Pelliccione*[2], *Corrado Romano*[3], *Livia Garavelli*[4], *Maria Lisa Dentici*[5], *Nicola Specchio*[5],

Paolo Alfieri[5], Paola Grammatico[6], Gabriele Trimarchi[4], Margherita Baldassarri[7], Alessandra Renieri[7], Roberta Milone[8], Flavio Faletra[2], Giuseppe Cossu[9], Giorgia Girotto[1,2], Marco Tartaglia[5], Paolo Gasparini[1,2], Maria Teresa Bonati[2]

[1]University of Trieste, Trieste, Italy, [2]IRCCS Burlo Garofolo, Trieste, Italy, [3]I.R.C.C.S. Oasi Maria SS., Troina, Italy, [4]AUSL Reggio Emilia, Reggio Emilia, Italy, [5]Ospedale Pediatrico Bambino Gesù, Roma, Italy, [6]Università La Sapienza, Roma, Italy, [7]University of Siena, Siena, Italy, [8]IRCCS Fondazione Stella Maris, Pisa, Italy, [9]Centro Medico di Foniatria, Padova, Italy.

Introduction: Neurodevelopmental disorders (NDDs) are characterized by genetics and phenotypic heterogeneity. Thus, Whole Exome Sequencing (WES) studies combined with a clinical evaluation can be a powerful approach maximizing molecular diagnostic yield. Heterozygous pathogenetic variants in *POGZ* gene have been associated to a syndromic NDD, including autism spectrum disorder (ASD), developmental delay (DD), intellectual disability (ID) and some dysmorphic facial features.

Material and methods: A multicentric, Italian WES data sharing has been carried out with the aim of providing a complete clinical and neurocognitive picture of patients with a similar phenotypic characteristic (a diagnosis of POGZ-related disorder), and negative to SNPs/CGH molecular karyotyping.

Result, new perspectives: All collected cases resembling a POGZ-related disorder (n = 13; 8 male) presented with ID (from mild to severe), a global DD, usually exhibited behavioural impairments and, in five cases, a diagnosis of ASD. The identified, novel pathogenetic or likely pathogenetic variant in POGZ include frameshift (5), stop (3), splicing (2) and missense (1) variants, mostly occurring *de novo*, except for a familiar case (3 subjects). Considering the unmet medical needs for most life-longing NDDs and recent evidences on the improvement of behavioural impairments in POGZ knock-in mice, we propose new care perspectives through the inhibition of glutamatergic signal and the mitigation of excitatory neurons. A recruitment of patients with POGZ-related disorder is dare for ongoing.

Conclusion: Combining depth clinical data with genomic finding, we highlighted, for the first time, the important role of *POGZ* gene in the pathogenesis of NDDs, opening new perspectives with therapeutic opportunities.

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P09.093.B AGO1 amino acid changes in neurodevelopmental disorders

***Clarisse Delvallee**, Sarah Baer, Léa Sanna, Valérie Skory, Jérémie Courraud, Nathalie Drouot, Damien Plassard, Jean-Louis Mandel, Amélie Piton*

IGBMC, Université de Strasbourg, Illkirch, France.

Introduction: AGO1 is a RNA-binding protein (RBP) from the Argonaute family involved in gene-silencing mediated by small non-coding RNA and additional processes regulating gene expression. We recently reported 28 patients with a neurodevelopmental disorder (NDD) harboring de novo amino acid changes in AGO1 (Schalk, Cousin et al, BioRxiv 2010). The role of AGO1 in neuronal cells, as well as the impact of these amino acid changes on its functions, remain to be elucidated.

Methods: We used human neural stem cells (hNSCs) to study the role of AGO1 and the consequences of its inactivation at molecular and cellular levels. In parallel, we overexpressed AGO1 mutant proteins in neuronal (Neuro2A) and non-neuronal (HeLa, HEK293) cells.

Results: We showed that AGO1 inactivation using siRNA did not alter the proliferation of hNSCs but impacts differentiation process in Neuro2A cells. Transcriptomic studies performed in hNSCs did not reveal any significant change at the mRNA level (except AGO1 itself) but identified significant changes in splicing events in several hundreds of genes, enriched in proteins related to DNA binding/transcription regulation. In parallel, AGO1 mutant proteins are stably expressed and localized in HeLa and HEK293 cells, but they show a different pattern of protein interactions compared to the wild-type AGO1 as revealed by IP-MS experiments.

Conclusion: The splicing and neurite outgrowth alterations identified in neural cells after AGO1 inactivation provide insight into how AGO1 dysfunction could impact brain development and can serve as read-out to test the effect of amino acid changes identified in patients with NDD.

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P09.094.C Genetic characterization of 274 patients with neurofibromatosis type 1: rare and diagnostically challenging co-occurrence of two variants in the same patient

***Rita Bastos Ferreira**[1,2], Susana Sousa[1,2], Ana Lopes[1,2], Ana Filipa Brandão[1,2], Sara Morais[1,2], Paulo Silva[1,2], Fátima Lopes[1,2], Alexandra Lopes[1,2], Cláudia Patraquim[3], Miguel Rocha[4], João Parente Freixo[1,2], Jorge Sequeiros[1,2], Jorge Oliveira[1,2]*

[1]CGPP-IBMC – Centro de Genética Preditiva e Preventiva, Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal, [2]i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal, [3]Serviço de Pediatria, Hospital de Braga, Braga, Portugal, [4]Serviço de Genética, Hospital de Braga, Braga, Portugal.

Neurofibromatosis type 1 (NF1) shows a wide phenotypic spectrum. Milder forms exhibit cutaneous and ophthalmological features: café-au-lait macules, axillary and inguinal freckles, cutaneous neurofibromas, and Lisch nodules. More severe phenotypes present a range of tumours (plexiform neurofibromas and optic nerve gliomas), variable neurological and cognitive features. NF1 is caused by variants in the neurofibromin (*NF1*) gene, arising *de novo* in ~50% of cases. A total of 536 patients with a clinical suspicion of NF1 were genetically tested. Variant screening in *NF1* was performed in all exonic regions, either by Sanger or next-generation sequencing (Ion Torrent). To detect large gene rearrangements in *NF1*, MLPA was used. A molecular diagnosis of NF1 was stablished for 274 patients (~51% of cases). Disease-causing variants encompass loss-of-function (n = 168), variants predicted to affect splicing (n = 49), missense variants (n = 29), inframe indels (n = 6), and large intragenic deletions or duplications (n = 6) or entire gene deletions (n = 16). One further patient with café-au-lait spots, epilepsy, developmental and psychomotor delay, harbored two *NF1* variants: a heterozygous variant c.731-2A>C co-segregating with NF1 in affected relatives, and a deletion of exon 19. Usually in such context, only the familial variant would be tested. However, as family history was not initially available, the entire *NF1* coding sequencing and MLPA was performed. This case shows the importance of a thorough *NF1* variants screening. As biallelic disfunction of *NF1* gene through a second-hit is critical for the development of additional clinical

features, precise genotyping is invaluable to establish an early diagnosis and for accurate genetic counselling.

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P09.095.D Estimated prevalence of Niemann-Pick type C disease in Quebec

Marjorie Labrecque[1,2], *Lahoud Touma*[1,3], *Claude Bhérer*[4], *Antoine Duquette*[1,3,5], *Martine Tétreault*[1,3]

[1]*CHUM Research Center, Montreal, QC, Canada,* [2]*Bioinformatics Program, Department of Biochemestry and Molecular Medecine, Université de Montréal, Montreal, QC, Canada,* [3]*Department of Neurosciences, Université de Montréal, Montreal, QC, Canada,* [4]*Department of Human Genetics, McGill University, Montreal, QC, Canada,* [5]*André-Barbeau Movement Disorders Unit, CHUM, Montreal, QC, Canada.*

Introduction: Niemann-Pick type C disease (NPC) is an autosomal recessive disease caused by mutations in the *NPC1* or *NPC2* genes. It has a large range of symptoms depending on age of onset, thus making it difficult to diagnose. In adults, symptoms appear mainly in the form of psychiatric problems. The prevalence varies from 0,35 to 2,2 per 100000 births depending on the country. The aim of this study is to calculate the estimated prevalence of NPC in Quebec to determine if it is underdiagnosed in the population.

Method: The CARTaGENE database regroups individuals between 40-69 years old with no known neurodegenerative disease. The blood RNA was available for 911 individuals and the blood DNA for 198 individuals. We used a bioinformatic pipeline on those individuals to extract the variants in the *NPC1/2* genes. The estimated prevalence was calculated using the Hardy-Weinberg Equilibrium.

Results: From the 452 variants identified, two were classified as pathogenic. The variant p.Pro543Leu was found in three heterozygous individuals (AF = 0,00148) that share a common haplotype. The variant p.Ile1061Thr was found in two heterozygous individuals (AF = 0,000984). Both variants are usually associated with an infantile or juvenile onset. The estimated prevalence calculated using those two variants is 1,21:100000 births.

Conclusions: Less than one case of NPC is diagnosed per year in Quebec which is equivalent to a minimal prevalence of 1,19:100000 births. The estimated prevalence found was 1,21:100000. The estimated result of is relatively close, meaning that NPC is probably not underdiagnosed in Quebec.

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P09.096.A Leukodystrophy in consanguineous Bedouin kindred caused by homozygous novel *NOTCH3* nonsense mutation

Amit Safran[1], *Regina Proskorovski-Ohayon*[1], *Ohad S. Birk*[1,2]

[1]*Morris Kahn Laboratory of Human Genetics, National Institute for Biotechnology in the Negev, Ben Gurion University, Beer Sheva, Israel,* [2]*Genetics Institute, Soroka Medical Center, Beer Sheva, Israel.*

Introduction: Leukodystrophies are a group of rare metabolic and genetic diseases affecting the white matter of the brain, spinal cord and often the peripheral nervous system. In the Bedouin community in Israel, consanguineous marriages are common, contributing to high rates of congenital malformations and genetic diseases. Leukodystrophy, presenting with severe mental retardation and epileptic seizures, was identified in a consanguineous Bedouin family.

Materials and Methods: Genome-wide linkage analysis combined with whole exome sequencing were performed to identify disease-causing variants. Exome data were narrowed down to a few variants using the Ingenuity Variant Analysis™ software and in-house WES data of 500 ethnicity-matched controls. Sanger sequencing and restriction fragment length polymorphism analysis was conducted to study recently found and novel variant segregation within the affected family.

Results: Based on analysis of gene expression databases and previous human and mouse studies, a homozygous nonsense mutation in *NOTCH3* (c.2221C>T; NM_ 000435.3; p.Q741*) was identified as the most probable disease-causing variant in the family.

Conclusions: *NOTCH3* (Notch Receptor 3) encodes a transmembrane protein involved in signaling pathways expressed during embryonic development. Mutations in *NOTCH3* have been previously identified as the underlying cause of a cerebral autosomal dominant disease named CADASIL. Moreover a single case of Leukodystrophy was identified with null *NOTCH3* mutation unexpectedly acting in recessive heredity. These findings highlight *NOTCH3* null mutations as a cause of autosomal recessive Leukodystrophy and will allow for carrier testing and early pre-implantation genetic diagnosis within the studied family and the larger Bedouin kindred.

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P09.097.B Don't take your (virtual) panels for granted!

Fulvio D'Abrusco[1], *Valentina Serpieri*[1,2], *Romina Romaniello*[3], *Filippo Arrigoni*[4], *Roberto Ciccone*[1], *Renato Borgatti*[5,6], *Enza Maria Valente*[1,2]

[1]*Dept. of Molecular Medicine, University of Pavia, Pavia, Italy,* [2]*Medical Genetics Unit, IRCCS Mondino Foundation, Pavia, Italy,* [3]*Neuropsychiatry and Neurorehabilitation Unit, Scientific Institute IRCCS Eugenio Medea, Bosisio Parini, Lecco, Italy,* [4]*Neuroimaging Lab, Scientific Institute IRCCS Eugenio Medea, Bosisio Parini, Lecco, Italy,* [5]*Dept. of Brain and Behavioral Sciences, University of Pavia, Pavia, Italy,* [6]*Child Neurology and Psychiatry Unit, IRCCS Mondino Foundation, Pavia, Italy.*

Recently, Next Generation Sequencing (NGS) has revolutionized the genetic diagnosis of rare diseases. The adoption of gene panels, clinical exome and whole exome sequencing (WES) increased the diagnostic yield even for complex syndromic patients, often leading to an expansion of the phenotypic spectrum of a given gene. Nonetheless, in the diagnostic setting, it becomes necessary to bioinformatically filter the data, restricting the analysis only to those genes compatible with the phenotype (so called "virtual panels"). For complex phenotypes, choosing the right panel may be tricky, leading to diagnostic mistakes. We report on two male siblings with corpus callosum agenesis, cerebellar hypoplasia, microcephaly, severe intellectual disability (ID), seizures, behavioral disorder, myopia, ataxia and peculiar face dysmorphisms. After WES, the analysis of a virtual panel of 381 genes implicated in corpus callosum abnormalities disclosed in both siblings a hemizygous variant in *PAK3*, a gene known to cause ID, epilepsy, corpus callosum agenesis and microcephaly. Yet, the extreme severity of ID and the peculiar dysmorphisms prompted a further analysis of a larger panel of ID-related genes. This showed a second hemizygous variant shared by both siblings in *FRMPD4*, a gene associated to ID, seizures, behavioral disorders and dysmorphic features. This example

confirms the advantage of a wider WES-based strategy over custom panels and illustrates the importance of a careful phenotyping before the analysis. Realistically, many complex cases labelled as "expansion of the phenotypic spectrum" of a given gene, may actually harbor mutations in two distinct genes, both concurring to the phenotype.

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P09.098.C Genetic analysis in a large cohort of patients with hereditary spastic paraplegia: diagnostic challenges

Sara Morais[1,2], *Ana Filipa Brandão*[1,2], *Ana Lopes*[1,2], *Rita Bastos-Ferreira*[1,2], *Susana Sousa*[1,2], *Paulo Silva*[1,2], *Fátima Lopes*[1,2], *Alexandra Lopes*[1,2], *Joana Damásio*[1,2,3], *José Leal Loureiro*[4], *Marina Magalhães*[3,5], *Miguel Leão*[6], *Cristina Costa*[7], *Ricardo Maré*[8], *Jorge Sequeiros*[1,2], *João Parente Freixo*[1,2], *Jorge Oliveira*[1,2]

[1]*CGPP-IBMC – Centro de Genética Preditiva e Preventiva, Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal,* [2]*I3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal,* [3]*Serviço de Neurologia, Hospital de Santo António, CHUP, Centro Hospitalar Universitário do Porto, Porto, Portugal,* [4]*Serviço de Neurologia, Centro Hospitalar de Entre o Douro e Vouga, Santa Maria da Feira, Portugal,* [5]*Serviço de Neurologia, Hospital Padre Américo, Centro Hospitalar do Tâmega e Sousa, Penafiel, Porto, Portugal,* [6]*Serviço de Genética, Centro Hospitalar de São João /Faculdade de Medicina da Universidade do Porto, Porto, Portugal,* [7]*Serviço de Neurologia, Hospital Professor Doutor Fernando Fonseca, Amadora, Portugal,* [8]*Serviço de Neurologia, Hospital de Braga, Braga, Portugal.*

Hereditary spastic paraplegias (HSPs) are a large group of neurodegenerative disorders, characterized by lower limb spasticity and weakness, but may include a range of other neurological and non-neurological symptoms. HSPs are genetically heterogeneous; at least 70 loci have been identified. Other genes, primarily associated to other phenotypes such as ataxia, have also been consistently associated with HSP.

At our centre, 139 HSP-related genes are routinely analysed, by single-gene tests or whole exome-based multigene panels, according to the clinical request. A total of 208 HSP patients (and 79 affected relatives) were successfully characterized: 118 by single-gene testing, 78 using multigene panels and 12 through larger NGS panels (e.g., clinical exome). Variants were identified at 33 different loci; *SPAST* (n = 89 cases) and *SPG11* (n = 25) were the most frequently involved. Also, interesting cases with variants in *SYNE1* or *ALS2* highlight the wide phenotypic spectrum associated with HSP. Multigene panels contributed to the identification of 66 cases harbouring variants of unknown clinical significance. A large number of patients (n = 90) carried a single (heterozygous) variant in genes associated to diseases with autosomal recessive inheritance. Besides new attempts to identify a missing pathogenic allele, functional studies may also clarify their causative role and, ultimately, provide a definitive diagnosis.

Our results demonstrate the importance of considering overlapping phenotypes and differential diagnosis for genes' selection for NGS panels design. Given the number of patients without any variant (n = 135), we presume that a significant number of genes related to HSPs are yet to be uncovered.

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P09.099.D *PRKN* analysis in Parkinson disease: two decades experience

Ana Filipa Brandão[1,2], *Sara Morais*[1,2], *Rita Bastos-Ferreira*[1,2], *Susana Sousa*[1,2], *Marina Magalhães*[3], *António Bastos Lima*[3], *José Alves Grilo Gonçalves*[4], *Leonor Correia Guedes*[5], *Alexandre Mendes*[3], *Ana Graça Velon*[6], *Carlos Sanchez Bueno*[7], *João Proença*[8], *Maria Manuela Manuela Costa*[9], *Ana Oliveira*[10], *Joaquim Ferreira*[5], *Mário Romero Blanco*[7], *Rui Araújo*[10], *Tânia Lampreia*[11], *João Parente Freixo*[1,2], *Jorge Sequeiros*[1,2], *Jorge Oliveira*[1,2]

[1]*CGPP-IBMC – Centro de Genética Preditiva e Preventiva, Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal,* [2]*i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal,* [3]*Serviço de Neurologia, Centro Hospitalar Universitário do Porto, Hospital de Santo António, Porto, Portugal,* [4]*Serviço de Neurologia, Centro Hospitalar e Universitário de Coimbra, EPE, Coimbra, Portugal,* [5]*Serviço de Neurologia, Hospital de Santa Maria, Centro Hospitalar de Lisboa Norte, EPE, Lisboa, Portugal,* [6]*Serviço de Neurologia, Hospital de São Pedro, Centro Hospitalar de Trás-os-Montes e Alto Douro, EPE, Vila Real, Portugal,* [7]*Serviço de Neurologia, Centro Hospitalar do Barlavento Algarvio, SA, Algarve, Portugal,* [8]*Serviço de Neurologia, Hospital Garcia de Orta, Lisboa, Portugal,* [9]*Serviço de Neurologia, Hospital das Forças Armadas - Pólo Porto, Porto, Portugal,* [10]*Serviço de Neurologia, Centro Hospitalar Universitário de São João/ Faculdade de Medicina da Universidade do Porto, Porto, Portugal,* [11]*Serviço de Neurologia, Hospital de Egas Moniz, Centro Hospitalar de Lisboa Ocidental, EPE, Lisboa, Portugal.*

Parkinson disease (PD) is a neurodegenerative disorder, characterized by rest tremor, muscle rigidity, bradykinesia, postural instability and dementia. Although predominantly sporadic, there are PD patients with autosomal recessive (AR) or dominant inheritance. Biallelic variants in the gene for parkin RBR E3 ubiquitin protein ligase (*PRKN*) is an important cause of AR PD. We describe the *PKRN* variants profile in a cohort of 524 patients with PD, tested between 2000-2020. *PRKN* analysis was performed by Sanger sequencing and/or MLPA, or a NGS multigene panel. A molecular diagnosis of PRKN-related PD was established in 63 patients, with 3 additional cases with variants of unknown clinical significance (VUS). A pathogenic variant in heterozygosity was also identified in 16 patients. Altogether, 29 *PRKN* variants have been identified: 7 missense, 3 affecting splice-sites, 2 small frameshift deletions and 1 insertion-deletion, plus 16 copy number variants (14 deletions, 2 duplications). Overall, 25 variants were classified as pathogenic or likely-pathogenic, whereas 4 are VUS. The obtained diagnostic yield (12%) is quite relevant considering the high clinical and genetic heterogeneity of PD. The c.155del change was the most frequently found variant (65% of cases). Surprisingly, however, large *PRKN* rearrangements were also identified in a significant proportion (54%) of patients. A definitive diagnosis of PD allows proper patient management and more precise genetic counselling of patients and families. As several gene-targeted therapies for PD have now reached the clinical trial stage (not yet the case for *PRKN*-related entity), the clinical utility of genetic testing for PD has expanded considerably.

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P09.100.A Over-mutated mitochondrial, lysosomal and TFEB-regulated genes in Parkinson disease

*Eulàlia Segur - Bailach[1], Olatz Ugarteburu[1], **Frederic Tort**[1], Laura Texidó[1], Celia Painous[2], Yaroslau Compta[2], Maria J Martí[2], Antonia Ribes[1], Laura Gort[1]*

[1]Secció d'Errors Congènits del Metabolisme-IBC, Servei de Bioquímica i Genètica Molecular, Hospital Clínic, IDIBAPS, CIBERER., Barcelona, Spain, [2]Parkinson's Disease & Movement Disorders Unit, Hospital Clínic/IDIBAPS/CIBERNED/European Reference Network for Rare Neurological Diseases (ERN-RND)/Institut de Neurociències, Universitat de Barcelona., Barcelona, Spain.

Introduction: Association of Parkinson's disease (PD) with mutations in genes involved in lysosomal and mitochondrial function has been reported. However, the involvement of other cellular mechanisms is unknown. We aim to identify novel genetic associations to better understand the pathogenesis of PD.

Material and Methods: We performed WES in a cohort of 33 PD patients and 30 age-matched controls. We searched for rare variants in 1665 genes: PD-causative (53), related to lysosomal function (128), TFEB-regulated (428) and Mitocarta 2.0 (1158). The variants were classified according to the ACMG criteria.

Results: We identified a burden of rare variants in genes associated to lysosomal or mitocondrial function in PD patients compared to controls, 45% vs 17% and 76% vs 39% respectively. In particular, we found an enrichment of mutations in genes encoding for proteins affecting the OXPHOS function and mtDNA maintenance. Interestingly, an important accumulation of rare variants in TFEB-regulated genes was observed in PD patients (85% vs 45%). The Z-score calculation using the European population database (GnomAD) showed an over-representation of particular variants in 36 of the analyzed genes. Remarkably, 11 of these genes have a mitochondrial function and 18 were TFEB-regulated genes.

Conclusions: We suggest the involvement of TFEB-regulated genes in the genetic susceptibility to PD. This is remarkable as TFEB factor has been reported to be sequestered inside Lewy Bodies, pointing to a role of TFEB in the pathogenesis of PD. Our data also reinforce the involvement of lysosomal and mitochondrial mechanisms in PD. Funding: ISCIII (PIE14/00061), CIBERER.

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P09.101.B Diagnostic yield of whole exome sequencing in early-onset and familial Parkinson's disease in the Balkans

*Aleš Maver[1], **Anja Kovanda**[1], Gaber Bergant[1], Natasa Teran[1], Irena Vrečar[1], Marija Brankovic[2], Milena Jankovic[3], Marina Svetel[2], Vladimir S. Kostic[2], Ivana Novakovic[4], Valentino Rački[5], Vladimira Vuletic[5], Borut Peterlin[1]*

[1]Clinical Institute of Genomic Medicine, Ljubljana, Slovenia, [2]Neurology Clinic, Faculty of Medicine, University of Belgrade, Belgrade, Serbia, [3]Neurology Clinic, CCS, Belgrade, Serbia, [4]Institute of Human Genetics and Neurology Clinic, Faculty of Medicine, University of Belgrade, Belgrade, Serbia, [5]Department of Neurology, University of Rijeka, Faculty of Medicine, Rijeka, Croatia.

Parkinson's disease (PD) is a common neurological disorder, hallmarked by progressive motor and autonomic dysfunctions and cognitive decline, with a typical onset after the age of 60. PD is multifactorial, with genetic variation in over 30 genes involved in PD risk, development, onset and progression. To assess the genetic component in development of PD in the Balkan population and to determine the diagnostic yield of whole exome sequencing (WES) in our clinical setting, we performed WES on a cohort of consecutive patients with early-onset (before age 50) and/or familial PD. We performed WES analysis of 100 patients with either early-onset and/or familial PD, consecutively referred from 2014 to 2021 to our center from Slovenia, Croatia and Serbia. The analysis was based on the Illumina Nextera Coding Exome targeting 37 Mb of exonic coding sequences and sequencing was performed on Illumina HiSeq 2500 in 2x100 reads paired-end sequencing mode. Variant interpretation was limited to a panel of PD associated genes. We determined pathogenic or likely pathogenic variants in PD associated genes of 15% of patients, while 26% of patients carried variants of uncertain significance (VUS) in PD-associated genes. The most commonly affected gene in our population was Glucosylceramidase Beta (GBA) (12/15 pathogenic or likely pathogenic variants, and 5/26 VUS). The results show diagnostic yield of WES in PD to be 15%, which is comparable to similar studies on other populations. GBA variants represent an important genetic contributor to early onset and/or familiar PD in the Balkan population.

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P09.102.C The study of the role of genetic risk factors in neurosychological disorders in Russian patients with Parkinson's disease

***Gulnara Akhmadeeva**[1], Irina Khidiyatova[2], Irina Gilyazova[2], Gulnaz Tayupova[3], Salavat Umutbaev[4], Azamat Baitimerov[3], Rim Magzhanov[1], Elza Khusnutdinova[2]*

[1]Bashkir State Medical University Ministry of Healthcare of the Russian Federation, Ufa, Russian Federation, [2]Institute of Biochemistry and Genetics Ufa Research Study Center, Ufa, Russian Federation, [3]Clinical Institute of Neurology and Rehabilitation National medical holding Limited Liability Company «Medstandart», Ufa, Russian Federation, [4]State budgetary healthcare institution Kuvatov Republican clinical hospital, Ufa, Russian Federation.

Introduction: More than 90% of patients with Parkinson's disease (PD) have various neuropsychological disorders: depression, anxiety and cognitive impairments. We assumed that there are genetic markers for neuropsychological disorders development in PD patients among polymorphic variants of genes of the dopaminergic and serotoninergic systems.

Materials and Methods: We investigated 357 PD patients from Republic of Bashkortostan. We used MMSE, the Beck depression inventory (BDI) and the state-trait anxiety inventory (STAI). The analysis of 18 SNPs of dopamine and serotonin receptors, serotonin transporter, monoamine oxidase B, catechol-O-methyltransferase, tryptophan hydroxylase and tyrosine hydroxylase genes was performed. The SPSS software was used for statistical analysis. A p-value <0.05 was considered statistically significant.

Results: Data on the influence of the rs6275*A/A genotype of the DRD2 gene on the development of anxiety in PD patients was obtained ($p = 0.041$). It has be shown that rs6280*T/T genotype of DRD3 gene can be a genetic marker of depression development in PD ($p = 0.023$), and shorter alleles (TH*6 and TH*7) of the (TCAT)n in the TH gene can be a genetic marker of depression with suicidal behavior ($p = 0.041$). A significant effect of the rs4680 polymorphism of the COMT gene on the MMSE indicators of the cognitive functions ($p = 0{,}019$) was established.

Conclusion: Our results show the possible influence of some polymorphic variants of the genes of the dopaminergic system on the development of certain neuropsychological disorders in Parkinson's disease. It is necessary to conduct more extensive

studies the larger sampling. The work was supported by RFBR grant #19-015-00331

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P09.105.B Analysis of DNM2, EPN2 & EXOC4 relative gene expression levels in peripheral blood from Parkinson's disease patients

Ekaterina Igorevna Semenova[1], *Margarita Maksimovna Rudenok*[1], *Anelya Khanlarovna Alieva*[1], *Alexey Vyacheslavovich Karabanov*[2], *Sergey Nikolaevich Illarioshkin*[2], *Petr Andreevich Slominsky*[1], *Maria Igorevna Shadrina*[1]

[1]*The Institute of Molecular Genetics of National Research Centre «Kurchatov Institute», Moscow, Russian Federation,* [2]*Research Center of Neurology, Moscow, Russian Federation.*

Introduction: Parkinson's disease (PD) is a widespread disorder of the nervous system. Because of the long prodromal period of the PD it is necessary to search for prognostic biomarkers. To date, there is evidence that impaired membrane transport can play an important role in the pathogenesis of PD; therefore, in our work we have analyzed changes in the relative mRNA levels of the DNM2, EPN2 and EXOC4 genes in the peripheral blood from treated and untreated patients with PD.

Materials and Methods: In the present work we have studied 2 groups of patients with PD and 2 comparison groups. Analysis of mRNA levels was performed using reverse transcription and real-time PCR with TaqMan probes.

Results: No significant changes in the expression of the studied genes were found in the group of untreated patients with PD. However, significant changes in the expression at the mRNA level for the DNM2 gene were obtained in the group of treated patients with PD.

Conclusion: Our results demonstrate that genes DNM2, EPN2 and EXOC4 are most likely not involved in the pathogenesis of the disease at the mRNA level in patients with early stage of PD. Therefore they probably cannot be used as prognostic biomarkers of PD. Changes in the expression of the DNM2 gene in treated patients with PD suggest that this gene is may be involved in processes associated with dopamine agonist therapy. This work was supported by the Russian Science Foundation (grants no. 20-15-00262).

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P09.106.C Dissecting the *HLA* locus in Parkinson's disease in Europeans

Eric Yu[1,2], *Aditya Ambati*[3], *Maren S. Andersen*[4,5], *Lynne Krohn*[1,2], *Mehrdad A. Estiar*[1,2], *Prabhjyot Saini*[1,2], *Konstantin Senkevich*[1,2], *Yuri L. Sosero*[1,2], *Ashwin A. K. Sreelatha*[6], *Jennifer A. Ruskey*[1,2], *Farnaz Asayesh*[1,2], *Dan Spiegelman*[1], *Mathias Toft*[4,5], *Marte K. Viken*[4,5], *Manu Sharma*[6], *Cornelis Blauwendraat*[7], *Lasse Pihlstrøm*[4], *Emmanuel Mignot*[3], *Ziv Gan-Or*[1,2]

[1]*McGill University, Montreal, QC, Canada,* [2]*The Neuro (Montreal Neurological Institute-Hospital), Montreal, QC, Canada,* [3]*Stanford University, Palo Alto, CA, USA,* [4]*Oslo University Hospital, Oslo, Norway,* [5]*University of Oslo, Oslo, Norway,* [6]*University of Tübingen, Tübingen, Germany,* [7]*National Institutes of Health, Bethesda, MD, USA.*

Introduction: Recent evidence from human and animal studies suggest that the immune system has an important role in Parkinson's disease (PD). Genetic evidence also nominated immune related genes such as *LRRK2*, *BST1*, and the human leukocyte antigen (*HLA*) locus. In this study, we performed the largest genetic analysis of the *HLA* region in PD.

Materials and Methods: We fine-mapped the *HLA* locus using 13,770 PD patients, 20,214 proxy-cases and 490,861 controls of European origin. We used genome-wide association studies (GWAS) data to impute *HLA* types and to performed multiple statistical analyzes, to examine the association of specific *HLA* types, haplotypes and amino acid changes with PD. We further performed conditional analyzes to identify specific alleles or genetic variants that drive the association with PD.

Results: Four *HLA* types were associated with PD after correction for multiple comparisons: *HLA-DQA1**03:01, *HLA-DQB1**03:02, *HLA-DRB1**04:01 and *HLA-DRB1**04:04. Haplotype analyzes followed by amino-acid analysis and conditional analyzes suggested that the association is protective and primarily driven by three specific amino acid polymorphisms present in most *HLA-DRB1**04 subtypes - 11V, 13H and 33H (OR = 0.87 95%CI = 0.83-0.90, $p < 8.23 \times 10^{-9}$ for all three variants). No other effects were present after adjustment for these amino acids.

Conclusions: Our results suggest that specific variants in the *HLA-DRB1* gene are associated with reduced risk of PD, providing additional evidence for the role of the immune system in PD. Although effect size is small and has no diagnostic significance, understanding the mechanism underlying this association may lead to identification of new targets for therapeutics development.

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P09.107.D A Patient with Parkinsonian-Pyramidal Syndrome due to a *TBK1* Mutation

Jelena Pozojevic[1], *Diego Santos-García*[2], *Teresa de Deus Fonticoba*[3], *Mónica Kurtis*[4], *Josep Gamez*[5], *Christine Klein*[1], *Mariana H. G. Monje*[6], *Ana Westenberger*[1]

[1]*Institute of Neurogenetics, Lübeck, Germany,* [2]*Department of Neurology, Complejo Hospitalario Universitario de A Coruña (CHUAC), A Coruña, Spain,* [3]*Section of Neurology, Hospital Arquitecto Marcide y Hospital Naval, Complejo Hospitalario Universitario de*

Ferrol (CHUF), A Coruña, Spain, [4]Department of Neurology, Hospital Ruber Internacional, Madrid, Spain, [5]Neurology Department, GMA Clinic, Autonomous University of Barcelona, Barcelona, Spain, [6]HM-CINAC, Hospital Universitario HM Puerta del Sur, Universidad CEU-San Pablo, Madrid, Spain.

Introduction: Pathogenic variants in *TBK1* cause amyotrophic lateral sclerosis/frontotemporal dementia spectrum neurodegenerative disorders. Occasionally, patients manifest cerebellar ataxia and a form of progressive supranuclear palsy and corticobasal syndrome/progressive nonfluent aphasia. We have identified the first patient with a pallidopyramidal syndrome due to a *TBK1* mutation.

Methods: Our patient underwent a series of clinical and neuroimaging examinations at two time points (2011 and 2017). Genetic investigations included gene-panel sequencing, long-range and quantitative PCR analyses.

Results: At the age of 40 years, our patient manifested with resting tremor, bradykinesia, and rigidity on his left side. Brain MRI was normal and a DaTSCAN revealed an asymmetric bilateral reduction of striatal tracer uptake. Thus, he was diagnosed with early-onset Parkinson´s disease. Within six years, the patient progressively developed a bilateral pyramidal syndrome, mild wearing-off, and dyskinesias. Another DaTSCAN and 18F-DOPA positron emission tomography showed striatal dopaminergic denervation with right-side predominance and cranio-caudal progression. He displayed no signs of lower motor neuron involvement or cognitive impairment. Gene-panel sequencing revealed a splicing variant c.701+1G>A in *TBK1*. No alternatively spliced *TBK1* mRNA forms were detected by long-range PCR. However, quantitative PCR revealed markedly decreased *TBK1* levels in the patient sample, as compared to three healthy controls.

Conclusions: Since the mutant *TBK1* mRNA is likely degraded by nonsense-mediated decay in our patient, haploinsufficiency is a conceivable disease mechanism. Our findings extend the phenotypic spectrum of pathogenic variants in *TBK1*, indicate a novel genetic cause of pallidopyramidal syndrome, and suggest screening for *TBK1* mutations in patients with atypical parkinsonism.

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P09.108.A De novo variants in the lysinedemethylase *PHF2* are associated with developmental delay, autistic behavior, and facial dysmorphism

Alexej Knaus[1], *Miriam Wojcik*[2], *Miriam Viktor*[3], *Katheryn Grand*[4], *Pedro A. Sanchez-Lara*[4], *Tzung-Chien Hsieh*[1], *Gaber Bergant*[5], *Wendy K. Chung*[6], *Alexa Geltzeiler*[6], *Erin Torti*[7], *Peter M. Krawitz*[1]

[1]*Institute for Genomic Statistics and Bioinformatics, University Hospital, Bonn, Germany,* [2]*Division of Newborn Medicine Division of Genetics and Genomics Boston Children's Hospital, Boston, MA, USA,* [3]*Center for Pediatrics, Devision for Neuropediatrics, University Hospital, Bonn, Germany,* [4]*Department of Pediatrics, Cedars-Sinai Medical Center, Los Angeles, CA, USA,* [5]*Centre for Mendelian Genomics, Clinical Institute of Genomic Medicine, UMC Ljubljana, Ljubljana, Slovenia,* [6]*Department of Pediatrics and Medicine, Columbia University Irving Medical Center, New York, NY, USA,* [7]*GeneDx, Gaithersburg, MD, USA.*

We report de novo missense variants in *PHF2* in three individuals causing a novel neurodevelopmental disorder characterized by developmental delay, expressive language delay, autistic behavior, stereotypy and facial dysmorphism. *PHF2* is a lysine-specific demethylase that has already been shown to promote memory consolidation in mice via CREB signaling, however, *PHF2* has yet not been implicated in congenital disorder.The first de novo variant affects the Jumonji-C domain (JmjC), c. 727G>C; p.(Asp243His), probably impairing demethylase activity. The other two de novo variants affect highly conserved amino acids in the plant homeodomain PHD, c.23G>C; p.(Cys8Ser) and c.125C>T; p.(Ala42Val). The cysteine residues of the PHD domain are crucial in forming a complex with Zn2+, p.(Cys8Ser) is predicted to compromise DNA binding; while p.(Ala42Val) lies in the highly conserved α-helix of the PHD domain.Further studies including gestalt matching and transcriptional profiling will explore the specific mechanisms implicated in the pathophysiology of *de novo* variants in *PHF2*.

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P09.109.B A variant in *PIGK* clears up the diagnosis of a patient with neurological disorder after 4 years been undiagnosed

Bárbara Fernández Garoz, *Verónica Cantarín Extremera, Anna Duat Rodríguez, Victor Soto Insuga, Elena González Alguacil, Juan José García Peñas, Luis González Gutiérrez-Solana, María Jiménez Legido, Beatriz Bernardino Cuesta, María Luz Ruiz-Falco Rojas, Nelmar Valentina Ortiz Cabrera*

Hospital Infantil Universitario Niño Jesús, Madrid, Spain.

Introduction: Glycosylphosphatidylinositol (GPI)-anchored proteins are glycolipids found on many blood cells and served to anchor other proteins to the cell surface. They are critical for embryogenesis, neurogenesis and cell signalling. Case presentation: We present a 5-year-old boy, who debuted with epilepsy at 7 months of age, with severe neurological manifestations: epileptic encephalopathy, progressive cerebellar atrophy, global developmental delay with axial hypotonia and spastic-dystonic disorder. A whole exome sequence was performed.

Results: This study reveals the presence of a variant in *PIGK* (c.748A>C; p.Thr250Pro) in homozygosity. Following the ACMG criteria and the relation between phenotype and genotype, we concluded that this variant is probably pathogenic and responsible for the neurodevelopmental disorder present in our patient.

Conclusions: *PIGK* gene encodes for an enzyme that is involved in GPI-anchor biosynthesis. Pathogenic variants in *PIGK* gene are related to neurodevelopmental disorder with hypotonia and cerebellar atrophy with or without epilepsy (MIM#618879). This syndrome has been recently described by Nguyen et al. (Nguyen et al, Am J Hum Genet, 2020). This disease is an autosomal recessive neurodevelopmental disorder characterized by global developmental delay, which is usually severe, accompanied by hypotonia with variable intellectual disability. Most patients develop early-onset seizures and movement disorder, such as ataxia or dysmetria associated with progressive cerebellar atrophy on brain imaging. These manifestations are consistent with the ones presented in our patient. On the other hand, being a recently described entity, the information available may not be completely consistent, and functional studies are needed.

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P09.110.C Functional analysis of *PLXNA1* variants in a novel neurodevelopmental syndrome with oculo-cerebral anomalies

Paulina Z. M. Kempe[1,2,3], *Öznur Yilmaz*[2], *Tobias T. Lindenberg*[2], *Jaya Punetha*[4,5], *Jeshurun C. Kalanithy*[2], *Haktan B. Erdem*[6], *Zeynep C. Akdemir*[4], *Ender Karaca*[7], *Tadahiro Mitani*[4], *Dana Marafi*[4], *Jawid M. Fatih*[4], *Shalini N. Jhangiani*[8], *Boris Keren*[9], *Julien Buratti Buratti*[9], *Perrine Charles*[9], *Caroline Nava*[9,10], *Davut Pehlivan*[4], *Jennifer E. Posey*[4], *James R. Lupski*[4,11,12,8], *Ben Odermatt*[2], *Heiko Reutter*[3], *Gabriel C. Dworschak*[1,2,3]

[1]*Institute of Human Genetics, Medical Faculty, University of Bonn, Bonn, Germany,* [2]*Institute of Anatomy, Medical Faculty, University of Bonn, Bonn, Germany,* [3]*Department of Pediatrics, University Hospital Bonn, Bonn, Germany,* [4]*Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA,* [5]*Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York City, NY, USA,* [6]*Department of Medical Genetics, University of Health Sciences, Diskapi Yildirim Beyazit Training and Research Hospital, Ankara, Turkey,* [7]*Department of Genetics, University of Alabama at Birmingham, Birmingham, AL, USA,* [8]*Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX, USA,* [9]*AP-HP, Hôpital Pitié-Salpêtrière, Département de Génétique, Paris, France,* [10]*Institut du Cerveau et de la Moelle épinière, Sorbonne Université, Paris, France,* [11]*Texas Children's Hospital, Houston, TX, USA,* [12]*Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA.*

PLXNA1 encodes for the transmembrane semaphorin receptor Plexin-A1 which plays a key role in axonal outgrowth and neuronal migration in the developing central nervous system (CNS). Previously, we reported 3 patients from 3 unrelated families with monoallelic missense variants and 8 patients from 5 unrelated families with biallelic variants in PLXNA1 (unpublished).

Knockdown of the zebrafish homologs plxna1a and plxna1b in zebrafish larvae (zfl) by antisense morpholinos (MO) was performed in wild-type and transgenic zfl Tg(-3.1ngn1:GFP). We performed rescue experiments by co-injection of MO together with human PLXNA1 mRNA. The eye development and axonal outgrowth of the zfl were analyzed in vivo using time-lapse microscopy. To assess the impact of the discovered alleles in PLXNA1, we will co-inject MO together with mRNA carrying the respective variants.

MO knockdown of plxna1a and plxna1b in zebrafish larvae resembled the human CNS and eye phenotype. The number of dorsal root ganglia and outgrowing axons as well as the eye diameter of the zfl was significantly reduced. While co-injection of MO together with human PLXNA1 mRNA resulted in a rescue of phenotype, the impact of human alleles in PLXNA1 is being tested in this model.

The zebrafish is a suitable model to study the role of PLXNA1 in CNS development and axonal outgrowth in vivo. Furthermore, this model allows the testing of (potentially) pathogenic variants in PLXNA1 in the context of a novel neurodevelopmental syndrome with oculo-cerebral anomalies.BonnNI:Q-614.2954(PZMK); DFG-RE 1723/5-1(HR); NHGRI and NHLBI grant to the Baylor-Hopkins-Center for Mendelian Genomics[UM1 HG006542](JRL); BONFOR:O-120.0001, Herbert-Reeck-foundation (GCD)

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P09.111.D Re-weighting hundreds of polygenic risk scores improves prediction accuracies of psychiatric and neurological disorders

Clara Albiñana, Florian Privé, Esben Agerbo, ISPYCH-Broad Consortium, Preben B. Mortensen, John McGrath, ***Bjarni Vilhjalmsson***

Aarhus University, Aarhus V, Denmark.

The genetic architecture underlying psychiatric and neurological disorders is remarkably complex and highly polygenic. Polygenic risk scores (PRS) trained on summary statistics from genome-wide association studies (GWAS) for these are therefore generally underpowered and capture only a fraction of the estimated heritable variation. However, many psychiatric and neurological disorders are genetically correlated with behavioral and cognitive traits that have been the focus of large GWAS. PRSs for correlated traits can therefore be used to improve prediction accuracy of related outcomes (Krapohl et al., Mol Psych 2018).

Here, we make use of 940 GWAS summary statistics for a variety of diseases and traits to train PRS for 50 psychiatric and neurological disorders and related subtypes in IPSYCH (Bybjerg-Grauholm et al., medRxiv 2020). We generated the 940 PRS using LDpred2-auto (Privé et al., Bioinformatics 2020), which does not require any validation data to fit hyper-parameters. We then use these as covariates for two multiPRSs prediction models, penalized regression and gradient boosted trees

We observe that multiPRS can drastically boost the prediction accuracy compared to marginal (single-outcome) PRS. For instance, the prediction R2 (adjusted for sex, age, and 20 genetic PCs) for the three most prevalent psychiatric disorders in the IPSYCH cohort ADHD, autism, and depression was 1.1%, 0.4%, and 2.6% when using the single-outcome PRS (excluding IPSYCH samples), and 9.1%, 4.0%, and 4.3% respectively using multiPRS. For some psychiatric diagnoses we obtain predictive PRS even though GWAS of these are not yet available.

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P09.112.A Correlation of *GAA* genotypes and enzymatic activity of acid-α-glucosidase among Hungarian Pompe disease patients

Aniko Gal[1], *Zoltan Grosz*[1], *Beata Borsos*[1], *Ildiko Szatmari*[2], *Agnes Sebok*[3], *Laszlo Javor*[4], *Veronika Harmat*[5], *Katalin Szakszon*[6], *Livia Dezsi*[7], *Eniko Balku*[8], *Zita Jobbagy*[9], *Agnes Herczegfalvi*[10], *Zsuzsanna Almassy*[11], *Levente Kerenyi*[12], *Maria Judit Molnar*[1]

[1]*Institute of Genomic Medicine and Rare Disorders, Semmelweis University, Budapest, Hungary,* [2]*First Department of Pediatrics, Semmelweis University, Budapest, Hungary,* [3]*Department of Neurology, University of Pecs, Pecs, Hungary,* [4]*Petz Aladár County Hospital, Győr, Hungary,* [5]*Department of Pediatrics, St. Rafael Hospital of Zala County, Zalaegerszeg, Hungary,* [6]*Department of Pediatrics, Faculty of Medicine, University of Debrecen, Debrecen, Hungary,* [7]*Department of Neurology, University of Szeged, Szeged, Hungary,* [8]*Department of Pediatrics, Andras Josa Teaching Hospital, Nyíregyháza, Hungary,* [9]*Department of Neurology, Bács-Kiskun County Hospital, Kecskemet, Hungary,* [10]*II. Department of Pediatrics, Semmelweis University, Budapest, Hungary,* [11]*Heim Pal Children's Hospital Budapest, Budapest, Hungary,* [12]*Department of Neurology, Szent György County Hospital, Szekesfehervar, Hungary.*

Introduction: Pompe disease is caused by the accumulation of glycogen in the lysosome due to deficiency of the lysosomal acid alpha-glucosidase (GAA) enzyme. The disease can be divided into two major groups based on age of onset 1.) late of onset Pompe disease (LOPD) (>12 months); 2.) infant onset Pompe disease (IOPD) (<12 months). In this study, we correlate the enzyme activity and the genetic alterations in the Hungarian patients with Pompe disease. **Patients and methods:** 24 patients with Pompe disease were enrolled. Enzymatic activity of acid-α-glucosidase was measured by mass spectrometry. The mutation analysis of *GAA* gene was performed with Sanger sequencing and MLPA methodology.

Results: 21 (87.5%) patients were classified as LOPD and 3 (12.5%) as IOPD. In this cohort 15 different pathogenic or likely pathogenic *GAA* mutations were detected in homozygous or compound heterozygous form. The most common alteration was the c.-32-13 T>G splice site mutation. By comparing the α-glucosidase enzyme activity of c.-32-13 T>G homozygous and compound heterozygous cases, the mean GAA activity in homozygous form is significantly higher than in the compound heterozygous cases. The lowest enzyme activity was found in cases where the c.-32-13 T>G mutation was not present.

Discussion: *B*ased on our study the localization of mutation and protein domain involvement correlated with the GAA activity. Our study provides valuable information on the Pompe disease genotype-phenotype correlation, which is expected to facilitate and improve genetic counseling of affected individuals and their family members.

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P09.113.B MINPP1 prevents intracellular accumulation of inositol hexakisphosphate and is mutated in Pontocerebellar Hypoplasia

Ekin Ucuncu[1], Karthyayani Rajamani[1], Miranda S. C. Wilson[2], Francesca Picco[1], Daniel Medina-Cano[1], Nami Altin[1], Nadia Bahi-Buisson[1], Catherine Fossoud[3], Fabienne Giuliano[4], Laurence Colleaux[1], Lydie Burglen[1], Joseph G. Gleeson[5], Nathalie Boddaert[1], ***Vincent Cantagrel***[1]

[1]Institut IMAGINE, Paris, France, [2]University College London, London, United Kingdom, [3]CHU-Lenval, Nice, France, [4]Centre Hospitalier Universitaire de Nice, Nice, France, [5]University of California San Diego, san diego, CA, USA.

Inositol polyphosphates are vital metabolic and secondary messengers, involved in diverse cellular functions. Therefore, tight regulation of inositol polyphosphate metabolism is essential for proper cell physiology. Here, we describe a very early-onset neurodegenerative syndrome caused by loss-of-function mutations in the multiple inositol polyphosphate phosphatase 1 gene (MINPP1). Patients from 6 families were found to have a distinct type of Pontocerebellar Hypoplasia with typical basal ganglia or thalami involvement on neuroimaging. We found that patient-derived and genome edited MINPP1$^{-/-}$ induced pluripotent stem cells (iPSCs) are not able to differentiate efficiently into neurons. MINPP1 deficiency results in an intracellular imbalance of the inositol polyphosphate metabolism. This metabolic defect is characterized by an accumulation of highly phosphorylated inositols, mostly inositol hexakisphosphate (IP_6), detected in HEK293, fibroblasts, iPSCs and differentiating neurons lacking MINPP1. IP_6 has strong chelating properties and is also known to be a cofactor for multiple enzymes involved in diverse functions including RNA editing and nuclear RNA export. In mutant cells, higher IP_6 level is expected to be associated with an increased chelation of intracellular cations, such as iron or calcium, resulting in decreased levels of available ions. These data highlight the critical role of MINPP1 and IP_6 regulation in human brain development and homeostasis.

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P09.114.C A biallelic frameshift indel in *PPP1R35* as a cause of primary microcephaly

Moez Dawood[1], *Gulsen Akay[1], Tadahiro Mitani[1], Jawid M. Fatih[1], Jaya Punetha[1], Jaya Punetha[1], Dana Marafi[1,2], Christopher M. Grochowski[1], Haowei Du[1], Angad Jolly[1], Zeynep Coban-Akdemir[1,3], Shalini N. Jhangiani[1], Jill V. Hunter[1,4], Davut Pehlivan[1,4], Jennifer E. Posey[1], Claudia M. B. Carvalho[1,5], Richard A. Gibbs[1], James R. Lupski[1,4]*

[1]Baylor College of Medicine, Houston, TX, USA, [2]Kuwait University, Safat, Kuwait, [3]The University of Texas Health Science Center at Houston, Houston, TX, USA, [4]Texas Children's Hospital, Houston, TX, USA, [5]Pacific Northwest Research Institute, Seattle, WA, USA.

Protein phosphatase 1 regulatory subunit 35 (*PPP1R35*) encodes a centrosomal protein required for recruiting microtubule-binding elongation machinery. *PPP1R35* interacts with several established primary microcephaly (MCPH) genes, and multiple *PPP1R35* model organism studies hypothesize *PPP1R35* as a candidate MCPH gene. Here, using exome sequencing and family-based rare variant analyses, we report a homozygous, frameshifting indel deleting the canonical stop codon in the last exon of *PPP1R35* [Chr7: c.753_*3delGGAAGCGTAGACCinsCG (p.Trp251Cysfs*22)] in a 3.7 Mb AOH block in a proband with severe MCPH (-4.3 SD at birth, -6.1 SD by 42 months), pachygyria, and global developmental delay from a consanguineous Turkish family. Droplet digital PCR confirmed mutant mRNA expression in fibroblasts. *In silico* prediction of the translation of mutant *PPP1R35* protein is expected to be elongated by 22 amino acids before encountering another stop codon. Aside from the proband family, this complex indel allele was absent in public databases (ClinVar, gnomAD, ARIC, 1000 genomes) and our in-house database of 14,000+ exomes including 1,800+ Turkish exomes. A comprehensive literature search for *PPP1R35* mutations yielded two probands affected with severe microcephaly (-15 SD and -12 SD) with the same homozygous indel from a single, consanguineous, Iranian family from a cohort of 404 predominantly Iranian families. The lack of heterozygous cases in two large cohorts representing the genetic background of these two families decreases suspicion for a founder allele. Finally, we propose two origin models for the same mutation in two different populations mediated by hairpin formation between complimentary CG rich segments flanking the stop codon.

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P09.115.D Novel variants identified in the rare undiagnosed families

Farzane Zare Ashrafi[1], *Fatemeh Peymani*[1], *Marzieh Mohseni*[1], *Sanaz Arzhangi*[1], *Mohammad R. Akbari*[2,3,4], *Hossein Najmabadi*[1,5], *Kimia Kahrizi*[1]

[1]*Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran, Islamic Republic of,* [2]*Women's College Research Institute, University of Toronto, Toronto, ON, Canada,* [3]*Dalla Lana School of Public Health, University of Toronto, Toronto, ON, Canada,* [4]*Institute of Medical Science, University of Toronto, Toronto, ON, Canada,* [5]*Kariminejad - Najmabadi Pathology & Genetics Center, Tehran, Iran, Islamic Republic of.*

Introduction: Rare disorders refer to a group of diseases that affect less than 200,000 people in the United States or less than 2,000 people in Europe. These diseases, although individually rare, are generally prevalent. It has been estimated that there are about 7000 rare genetic diseases that the genetic cause is recognized for about half. Many of them affect the nervous system; causing some common phenotypes including intellectual disability, autism, epilepsy, ataxia, muscular dystrophy and neuropathy. Recent advances in molecular approaches like next-generation sequencing (NGS) make it possible to reach an accurate molecular and subsequently clinical diagnosis for patients with presumed rare genetic disorders who remained previously undiagnosed.

Materials and methods: 20 families with an undiagnosed rare genetic disorder were studied by whole-exome sequencing (WES).

Results: 18 causative variants in genes (*LARP7(2), TWNK, L2HGDH, UNC80, ANO10, TRRAP, STAMBP, MAP3K20, ZNF142, TAF2, WDR81, CHRNE, EFTUD2, SPTBN2*, *MAN1B1*, *BCL11B* and *DIAPH3*) have been detected by WES in 15 families but no candidate variants were identified in 4 families and one family is under study by using whole-genome sequencing (WGS). In total, we identified 12 single variants in 12 patients which one of them is a known copy number variation (CNV) reported for hearing impairment.

Conclusions: NGS approaches can significantly improve diagnostics of rare genetic disorders. We are reporting *BCL11B* variant as a de novo mutation as it has been reported previously in other studies. This study was supported by INSF (Iran National Science Foundation) with grant number 960111200 to Kimia Kahrizi.

F. Zare Ashrafi: None. **F. Peymani:** None. **M. Mohseni:** None. **S. Arzhangi:** None. **M. Akbari:** None. **H. Najmabadi:** None. **K. Kahrizi:** None.

P09.116.A Mapping the GBA1 gene rare variants and their effects in a Hungarian Parkinson's Disease Cohort

Tamás Szlepák[1], *Annabel Plamen Kossev*[1], *Dóra Csabán*[1], *Anett Illés*[2], *Beáta Borsos*[1], *Péter Balicza*[1], *Zoltán Grosz*[1], *Annamária Takáts*[1], *Péter Klivényi*[3], *Mária Judit Molnár*[1]

[1]*Semmelweis University, Budapest, Hungary,* [2]*Pentacore Laboratories and Semmelweis University, Budapest, Hungary,* [3]*University of Szeged, Szeged, Hungary.*

Introduction: Parkinson's Disease (PD) is a neurodegenerative disorder associated with genetic alterations in cc. 7-15% of the cases. *GBA1* is considered one of the major genetic risk factor for PD. The frequency of its rare variants in distinct ethnic populations and the penetrance of these variants in individuals can be different. Therefore, the genetic burden of *GBA1* variants and the exact genotype-phenotype correlation is still a hot topic today.

Methods: Patients from the institute's biobank (NEPSYBANK) were selected for the study (N = 116). The enrollment criteria included the diagnosis of PD, early onset and/or positive family history for PD, and negative findings for other PD related genes. The occurrence of *GBA1* variants were identified by either Sanger or NGS. Pathogenicity of the variants was determined according to the ACMG guidelines.

Results: *GBA1* rare variants were detected in 21 PD patients. The most frequent mutations are the T408M(n = 12) and E365K(n = 4). We identified 5 further rare variants (n = 1-2), two patients had 2 heterozygous variants. Most of the patients with T408 had cognitive decline and tremor dominant PD, with E365K depression beside the typical PD signs. Two patients had atypical PD with spasticity and/or pyramidal signs. Cognitive deficit was present in 35% of the *GBA1* positive cases.

Conclusion: *GBA1* rare variants were present in 18,1% of our PD cohort. The early identification of these patients is important, since they may have targeted causative treatment by using the substrate reduction treatment or lysosomal exocytosis stimulator. This study was supported by KTIA_13_NAP-A-III/6; KTIA_NAP and with the FIKP program

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P09.117.B Genome-Wide Association and Whole Exome Sequencing Studies reveal a Novel Candidate Locus for Restless Legs Syndrome

Ufuk Ergun[1], *Bahar Say*[1], *Sezen Guntekin Ergun*[2], *Ferda Percin*[3], *Levent Inan*[4], *Sukran Kaygisiz*[5], *Pınar Gelener Asal*[6], *Buket Yurteri*[2], *Maksim Struchalin*[7], *Dmitry Shtokalo*[8], ***Mehmet A. Ergun***[3]

[1]*Kırıkkale University, Kırıkkale, Turkey,* [2]*Hacettepe University, Ankara, Turkey,* [3]*Gazi University, Ankara, Turkey,* [4]*Ankara Training and Research Hospital, Ankara, Turkey,* [5]*Ordu University Education and Research Hospital, Ordu, Turkey,* [6]*Near East University, Lefkosa, Cyprus,* [7]*AcademGene Ltd, Novosibirsk, Russian Federation,* [8]*A.P. Ershov Institute of informatics systems, Moscow, Russian Federation.*

The restless legs syndrome (RLS) is a common heritable neurologic disorder which is characterized by an irresistible desire to move and unpleasant sensations in the legs. We aim to identify new variants associated with RLS by performing genome-wide linkage and subsequent association analysis of forty member's family with history of RLS. We found evidence of linkage for three loci 7q21.11 (HLOD = 3.02), 7q21.13-7q21.3 (HLOD = 3.02) and 7q22.3 (HLOD = 3.09). Fine-mapping of those regions in association study using exome sequencing identified SEMA3A (p-value = 8.5·10-4), PPP1R9A (p-value = 7.2·10-4), PUS7 (p-value = 8.7·10-4), CDHR3 (p-value = 7.2·10-4), HBP1 (p-value = 1.5·10-4) and COG5 (p-value = 1.5·10-4) genes with p-values below significance threshold. Linkage analysis with subsequent association study of exome variants identified six new genes associated with RLS mapped on 7q21 and q22.

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P09.119.D Prevalence of Spinocerebellar ataxia type 1 in Yakutia (Russia)

Afanasy I. Fedorov[1], *Aitalina L. Sukhomyasova*[1], *Irina A. Nikolaeva*[2], *Anastasia A. Maksimova*[1], *Aiaan V. Ivanov*[1], *Polina I. Golikova*[1], *Nadezhda R. Maksimova*[1]

[1]*North-Eastern Federal University named after M.K. Ammosov, Yakutsk, Russian Federation,* [2]*Medical Genetic Center, Republican Hospital №1 – "National Medical Center", Yakutsk, Russian Federation.*

Introduction: Spinocerebellar ataxia type 1 (SCA1) refers to polyglutamine diseases. It is a progressive and late-manifestation neurodegenerative disorder.

Material and Methods: We analyzed SCA1 Register data of the Medical Genetic Center of the Republican Hospital No. 1 (National Center of Medicine). Monitoring of carriers of the SCA1 mutation covers a 20-year period.

Results: During the monitoring period, the prevalence of SCA1 in Yakutia increased from 35 to 77.6 cases per 100,000 Yakuts. Currently, 532 carriers of mutation have been registered, of which 376 with clinical manifestations. The SCA1 mutation in Yakutia are distributed over the following geographic foci - Northern (Indigirka River), Central (Lena-Aldan interfluve) and Southwestern (Vilyui and Lena rivers). Currently, the expansion of the boundaries of the Northern and Southwestern focuses of mutation has been established. New isolated cases of mutation carriage are also recorded in the southern regions. The accumulation of the mutation continues in the Central focus. The median number of CAG repeats for the mutant allele is 46 repeats. The median age of carriers is 43 years old.

Conclusion: The increase in the prevalence of the studied mutation can be explained by the improvement of molecular genetic diagnostic methods. Currently, among the population of Yakutia, the accumulation and spread of the SCA1 mutation continues. This requires further improvement of methods for the prevention of this hereditary disease. Research is part of the project FSRG-2020-0014 "Arctic Genomics: epidemiology, heredity and pathology".

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P09.120.A Oxytocin receptor gene and CD38 gene polymorphisms association with social functioning in schizophrenia

Vera Mikhailova[1,2], *Victoria Plakunova*[1], *Tatiana Lezheiko*[1]

[1]*The Mental Health Research Center (MHRC), Moscow, Russian Federation,* [2]*Research Centre for Medical Genetics, Moscow, Russian Federation.*

Introduction: Social adaptation is the main ability for any schizophrenia patient and its family to judge whether the therapy is working or not, improving of social functioning is the mail goal to reach for healthcare professionals. Oxytocin plays an important role in social behavior, multiple *OXTR* polymorphisms has been observed to be associated with autism, OCD, bipolar disorder.

Materials and Methods: Social functioning was assessed with the Personal and Social Performance (PSP) scale in 933 patients (women 412), aged 36,5 ± 12,3 years, with ICD-10 diagnosis of schizophrenia or schizoaffective psychosis. Patients were stratified into moderate and good or poor (n = 233) social functioning groups. Genotyping was performed with HRM (High Resolution Melt) method. Allele frequencies were aligned with Hardy-Weinberg equation (p>0,05).

Results: Logistic regression models were estimated to examine the association between social functioning, SNPs and environmental factors such as birth complications, parental alcoholism, history of abuse in childhood or puberty etc. Adjusted models included as significant interaction between CD38(rs3796863) and father's alcoholism ($p < 0,05$); OXTR(rs53576) interaction with childhood difficulties ($p < 0,001$). Patients with CD38(rs3796863) CC genotype who have reported father's alcoholism were less likely to be in moderate and good social performance group (OR = 0,26, 95%CI 0,20-0,34). OXTR rs53576 G allele carriers with a history of abuse were also less likely to be in moderate and good social performance group (OR = 0,06, 95%CI 0,01-0,30).

Conclusion: Oxytocin pathway polymorphisms were found to be associated with social functioning and environmental factors in schizophrenia patients, CD38 rs3796863 C and OXTR rs53576 G being risk alleles.

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P09.121.B Developing expression system for evaluation of *SCN1A* splicing alterations

Peter Sparber, *Kseniya Davidenko, Margarita Sharova, Alexandra Filatova, Mikhail Skoblov*

Research Centre for Medical Genetics, Moscow, Russian Federation.

Introduction: Heterozygous pathogenic variants in the *SCN1A* gene is considered to be one of the most common causes of childhood-onset epilepsies. To date almost 200 pathogenic variants are annotated as splice-affecting, many of which locate outside of the canonical splice sites dinucleotides. However, only few were confirmed to disrupt splicing by a valid splicing assay. Here, we develop a robust splicing assay for functional characterization of intronic and exonic variants covering all protein coding exons of the SCN1A gene.

Material and methods: Splicing effects were predicted using HSF3.1, MaxEntScan and SpliceAI. Mini-, midi- and maxi-genes plasmids with coding *SCN1A* exons were constructed based on pSpl3-Flu2 splicing vector. The intronic/exonic variants of interest were introduced by site-directed mutagenesis. The plasmids were transfected into HEK293 cells using calcium phosphate transfection method. Splicing pattern was evaluated using RT-PCR with following Sanger sequencing.

Results: Splicing vectors with different genomic context, several promoters of varying strength, containing different exons were created in order to reproduce the wild-type splicing pattern of the *SCN1A* gene. 24 previously published intronic variants and one novel missense variant were then testes for splicing alteration. The most common splicing change was exon skipping (37.5%) followed by complex splicing changes (29.2%) and cryptic acceptor site activation (16.6%). 4 tested variants showed no splicing change, although being reported as pathogenic or likely pathogenic in the literature.

Conclusion: A splicing assay for the *SCN1A* gene was created. Testing of more than 20 variants confirmed the importance of functional analysis for proper variant annotation.

P. Sparber: None. **K. Davidenko:** None. **M. Sharova:** None. **A. Filatova:** None. **M. Skoblov:** None.

P09.122.C Proteomics of the dentate gyrus reveals semantic dementia specific biology

Merel O. Mol[1], *Shamiram Melhem*[1], *Suzanne S. M. Miedema*[2], *Harro Seelaar*[1], *Netherlands Brain Bank*[3], *August B. Smit*[2], *John C. van Swieten*[1], *Jeroen G. J. van Rooij*[1]

[1]*Department of Neurology, Erasmus Medical Center, Rotterdam, Netherlands,* [2]*Center for Neurogenomics and Cognitive Research, VU University, Amsterdam, Netherlands,* [3]*Netherlands Institute for Neuroscience, Amsterdam, Netherlands.*

Introduction: Semantic dementia (SD) is a subtype of frontotemporal dementia (FTD) characterized by impaired word comprehension and semantic memory. The consistent neuropathological diagnosis is FTD-TDP subtype C, with TDP-43 protein aggregates in the temporal cortex and dentate gyrus of the hippocampus. Despite this striking clinicopathological concordance, the pathophysiological mechanisms remain largely unknown.

Materials and Methods: We assessed the relative protein abundance changes in laser capture micro-dissected dentate gyrus of 15 SD patients and 14 age- and sex-matched non-demented controls using a label-free quantitative proteomics approach. We identified proteins and biological pathways that might be uniquely altered in SD, by comparing to 9 other large FTD and Alzheimer's Disease (AD) proteomics datasets. Validation experiments on selected candidate proteins are ongoing, including immunoblotting.

Results: 145 of all 2414 detected proteins showed differential abundance (FDR < 5%) in SD patients. 73 proteins were observed in at least 2 other proteomics studies in FTD/AD. The remaining 72 proteins were regarded as potentially SD specific and selected for further analyses. Functional enrichment revealed an overrepresentation of the cell-cell adherens junction and the cadherin-catenin complex, represented by multiple upregulated proteins within this complex, including CDH2/N-cadherin, CTNNB1/ß-catenin, and JUP/plakoglobin.

Conclusions: Our findings indicate an SD specific upregulation of cell adhesion proteins constituting the cadherin-catenin complex at the synaptic membrane. Validation of several proteins by immunoblotting is currently being performed. This study contributes to an improved understanding of the disease processes in SD, and demonstrates the value of quantitative proteomics to differentiate the pathophysiological mechanisms of specific subtypes of dementia.

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P09.123.D Clinical and genetic characteristics of two patients from Russia with SESAME syndrome due to mutations of the *KCNJ10* gene

Natalia Semenova*, Olga Schagina, Andrey Marakhonov*

Reseach Center for Medical Genetics, Moscow, Russian Federation.

Introduction: SESAME syndrome (seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalance) is caused by homozygous or compound heterozygous mutation in the *KCNJ10* gene. Generally, patients have severe symptoms of the disease.

Materials and methods: We present two children of the same age (10 years old) with SESAME syndrome and different clinical pictures. Both of them were from non-consanguineous parents with no significant family history. Patient 1 is male and has seizures, mental retardation, and severe electrolyte imbalance. He could not sit and walk by himself. He could understand spoken language but could not speak. The serum K-level was 2.3-2.5 mmol/l (normal 4.1-5.3 mmol/l). Patient 2 is a female with ataxia, intention tremor, and dyskinesia. She studies in an ordinary school with high grades. Her speech has elements of chanting. At the age of 4y11m, she had febrile seizures and started taking antiepileptic therapy. Without therapy, ataxia and tremor increase and she has a headache. The details of their symptoms are presented in the Table.

Results: The diagnosis was confirmed by WES in Patient 1 and WGS in Patient 2. We identified novel variants in the *KCNJ10* gene in the compound heterozygous state: c.322T>C/c.643G>A and c.148C>T/c.925T>A. The variants were verified by Sanger sequencing. Parents and siblings are healthy and heterozygous for either mutant allele.

Conclusions: We describe patients with the different phenotypes of SESAME syndrome.

	Patient 1	Patient 2
Gender	m	f
Mutations	c.322T>C / c.643G>A	c.148C>T / c.925T>A
Short stature	+	-
Hearing loss, sensorineural	ND	-
Salt craving	ND	-
Renal potassium wasting	+	-
Seizures	+	+
Psychomotor delay	+	-
Mental retardation	+	-
Ataxia	+	+
Ability to walk	-	+
Poor speech development	+	-
Intention tremor	+	+
Metabolic alkalosis	-	-
Hypokalemia	+	-
Increased plasma renin	+	-
Increased plasma aldosterone	+	-
Onset of seizures	3m	4y11m
Brain MRI	cortical-subcortical brain atrophy	subatrophic changes in the brain
EEG monitoring	epileptic activity in the occipital lobes	no typical epileptiform discharges

N. Semenova: None. **O. Schagina:** None. **A. Marakhonov:** None.

P09.124.A Discrepancy in phenotypes between *SHANK2* deletion and nonsense mutations in hiPSC-derived neural stem cells

Flavia-Bianca B. Cristian[1], *Carsten Sticht*[2], *Ralph Röth*[1,3], *Gudrun A. Rappold*[1], *Simone Berkel*[1]

[1]*Department of Human Molecular Genetics, Institute of Human Genetics, Heidelberg University Hospital, Heidelberg, Germany,* [2]*Core Facility Next Generation Sequencing, Medical Faculty Mannheim,*

Heidelberg University, Mannheim, Germany, [3]nCounter Core Facility, Institute of Human Genetics, Heidelberg University Hospital, Heidelberg, Germany.

SHANK2 mutations have been consistently associated with autism spectrum disorders (ASD). However, the specific consequences of these mutations at the molecular/cellular level, including the cell types/developmental stages that are crucially relevant for the phenotype, remain unclear. A fundamental question is whether the affected processes are limited to synapse formation/function, or if the pathobiology of the disorder starts earlier, either in differentiating neurons or already in neuronal progenitors. We have reprogrammed fibroblasts from a trio – two neurotypical parents and their daughter diagnosed with ASD, harboring a *de novo* 120 kb deletion in *SHANK2* – into induced pluripotent stem cells (iPSCs), and subsequently differentiated them into neural stem cells (NSCs). For comparison, we used four additional iPSC lines: one derived from a patient with a heterozygous nonsense mutation in *SHANK2* (R841X), a CRISPR/Cas9-engineered homozygous *SHANK2* knockout line, and their respective isogenic controls. Interestingly, only the NSCs derived from the deletion patient presented a strikingly different morphology, whereas the other *SHANK2*-deficient lines were indistinguishable from wildtype cells. Moreover, the *SHANK2* deletion NSCs showed a tendency towards premature differentiation into neurons. The discrepancy between the phenotypes, coupled with comparable SHANK2 levels in the deletion patient and controls, suggests that the altered phenotype might be caused by genetic factors independent of SHANK2 expression. In fact, this *SHANK2* deletion removes not only exon 16, but also a large portion of the adjacent intron. This region harbors numerous enhancers hypothesized to be relevant for general neural differentiation, and their deletion could contribute to the observed phenotype.

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P09.125.B Three novel heterozygous variants in the *MACF1, POLA1* and *TOP3B* genes: a new phenotype associated with the *TOP3B* gene?

Betül Gerik-Celebi[1], *Hilal Aydin*[2]

[1]*Department of Medical Genetics, Balikesir Ataturk City Hospital, Balikesir, Turkey,* [2]*Department of Pediatric Neurology, Balikesir University Faculty of Medicine, Balikesir, Turkey.*

Introduction: Epilepsy is a multifactorial and heterogeneous disorder that occurs mainly due to structural, metabolic, immunological and genetic reasons. Epilepsy can be seen as part of the clinical spectrum of chromosomal abnormalities, single gene disorders, microdeletion and duplication syndromes, with different inheritance patterns, pathophysiology, and accompanying dysmorphic and / or non-dysmorphic findings. It is extremely important to diagnose patients with epilepsy, to know additional preventable findings, to recover completely with treatment in some of them, and to prevent the emergence of individuals with similar findings with prenatal preimplantation genetic diagnosis.

Materials and Methods: Whole exome sequencing was performed using Illumina HiSeq 4000 instrument on four different patients who presented with epilepsy and dysmorphic symptoms. We used sanger sequencing for mother and father carrier screenings.

Results: As a result of whole exome sequencing analysis NM_012090.5: c.3133G>T: p.Val1045Leu, NM_016937.4: c.1436C>T: p.Thr479Ile, NM_003935.5: c.2018T>A: p.Leu673Gln novel heterozygous variants were detected in the *MACF1, POLA1* and *TOP3B* genes, respectively, and the homozygous *SLC13A5* gene: c.425C>T: p.Thr142Met mutation. In the analysis of POLA1 gene segregation, it was observed that the variant was inherited from the mother. Parent studies of other variants continue.

Conclusions: In this study, we found two novel heterozygous variants associated with the extremely rare diseases Lissencephaly 9 with complex brainstem malformation (LIS9 [MIM no: 618325) and Van Esch-O'Driscoll syndrome (VEODS [MIM no: 301030]). Although the *TOP3B* gene is not defined as the genotype of a particular disease in OMIM, its pathogenic variants have been associated with epilepsy, cognitive and behavioral disorders.

B. Gerik-Celebi: None. **H. Aydin:** None.

P09.127.D Familywise whole-genome linkage analysis of specific language impairment (SLI) identifies novel loci and replicates previous findings

Erin M. Andres[1], *Kathleen Kelsey Earnest*[1], *Shelley D. Smith*[2], *Mabel L. Rice*[1], *Muhammad Hashim Raza*[1]

[1]*University of Kansas, Lawrence, KS, USA,* [2]*University of Nebraska Medical Center, Omaha, NE, USA.*

Introduction: Individuals with specific language impairment (SLI) are often slow to talk and their performance on multiple measures of language remains below their age-matched peers throughout development, despite no known cause and typical non-verbal intelligence. Twin and family studies indicate genetic factors are involved in SLI. Although there are numerous reports of genomic regions and genes, the underlying causal pathways of SLI have not been explained.

Materials and Methods: We used SNP genotyping data from six families (N = 60) followed longitudinally, all with multiple members who have SLI, to perform genome-wide parametric linkage analysis. SLI phenotype status was categorically defined based on the lowest score across time points on an age-appropriate standardized omnibus language measure. Behavioral information was available for both parents of each proband.

Results: Suggestive linkage at 14q11.2-q13.3 in family 489 (LOD = 2.4) replicated a previous region of interest. Additionally, a three-branch extended family (315) showed linkage to a novel SLI locus at 15q24.3-25.3 (LOD = 3.06), while another family (300) showed suggestive linkage at 4q31.23-q35.2 (LOD = 2.4). All the highest LOD scores were identified under a recessive mode of inheritance. The other three families did not reveal suggestive linkage.

Conclusions: This initial study of families from the KU SLI cohort indicates the continued importance of family-based investigation of complex disorders, like SLI. The results will serve as the foundation for targeted follow-up inquiry to identify shared gene effects among families segregating SLI. Funding: NIDCD (T32DC000052 and R01DC001803)

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P09.128.A Identification of enhancer regions to expose novel genetic causes of spinocerebellar ataxia

***Fatemeh Ghorbani**, Eddy N. de Boer, Kim A. de Lange, Dineke S. Verbeek, Helga Westers, Cleo C. van Diemen*

University Medical Center Groningen, Groningen, Netherlands.

Introduction: Spinocerebellar ataxias (SCAs) are a group of genetically heterogeneous, dominantly inherited ataxias. Although 38 SCA genes are known, approximately 25% of patients remain genetically undiagnosed upon testing of the coding regions of SCA genes for variations. We hypothesize that variants in the regulatory regions of SCA genes might contribute to the disease in some genetically undiagnosed patients. Here, we aim to identify the yet unknown cerebellar enhancers of SCA genes with the ultimate goal to screen these regions for variations in patients.

Materials and methods: We selected four genes, *TBP, ATXN3, ATXN1* and *ITPR1*, for enhancer identification in neuroblastoma SH-SY5Y cells and healthy human cerebellum using 4C-seq. Putative enhancers were prioritized for follow-up studies by overlapping cerebellar/SH-SY5Y 4C-seq data with publicly available SH-SY5Y/cerebellar ATAC-seq, ChiP-seq and Dnase-seq data. Next, these regions will be validated with in vitro luciferase assays and screened for genetic variations in 500 genetically undiagnosed SCA patients.

Results: The 4C-seq data showed 2, 3 and 1 shared putative enhancer regions between cerebellum and SH-SY5Y for *TBP, ATXN3, ATXN1*, respectively, after prioritization with ATAC-seq, ChiP-seq and Dnase-seq data. Additionally, 2, 1 and 7 putative enhancer regions were unique for the cerebellum for *ATXN3, ATXN1* and *ITPR1*, respectively.

Conclusions: Putative cerebellar and SH-SY5Y enhancer regions are quite similar for three of the four SCA genes now tested. The identification of enhancers will help to understand how the expression of SCA genes is regulated in the human cerebellum and whether variation in these regions may lead to disease.

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P09.130.C Brain region specific effects on the expression of glucocorticoid receptor-regulated genes

***Nathalie Gerstner**[1,2,3], Anthodesmi Krontira[1,3], Janine Knauer-Arloth[1,2], Elisabeth B. Binder[1,4]*

[1]Max Planck Institute of Psychiatry, Munich, Germany, [2]Institute of Computational Biology, Helmholtz Zentrum München, Munich, Germany, [3]International Max Planck Research School for Translational Psychiatry, Max Planck Institute of Psychiatry, Munich, Germany, [4]Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, GA, USA.

Functional variants increasing the risk for psychiatric disorders alter the transcriptional response of glucocorticoid receptor (GR) target genes. The GR-regulated transcripts form brain-region specific co-expression networks whose structure is affected by different behavioral stressors throughout development in mouse models. To further study the regional specificity of brain response to GR activation, we stimulated with glucocorticoids or vehicle 30 mice for four hours, isolated eight distinct brain regions (prefrontal cortex-PFC, amygdala, cerebellum-CER, paraventricular nucleus of the hypothalamus, dorsal and ventral cornu ammonis 1 and dorsal and ventral dentate gyrus) and performed RNA sequencing. Profiling patterns of differential gene expression (DE) in each of the brain regions, we observed a strong response of the PFC (245 unique differentially expressed (DE) genes, FDR 10%) and the CER (176 unique DE genes), while 172 genes were differentially expressed across all regions. Furthermore, we employed a prior-guided gene expression network inference strategy to analyze GR-regulated co-expression patterns within and across brain regions. We map the identified transcriptional profiles to a circuit-level in order to get a better understanding of the molecular mechanisms involved in the brain response to stress.

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P09.131.D STXBP1 splicing variant caused developmental delay, hypotony and dysmorphic features without epilepsy

***Ahmet Yesilyurt**[1], Eyyup Uctepe[2], Sait Tumer[1], Nisa Esen[1]*

[1]Acibadem Labgen Genetic Diagnosis Center, Istanbul, Turkey, [2]Acıbadem Labmed/Ankara Tissue Typing Laboratory, Ankara, Turkey.

STXBP1 gene mutations are among the most common mutations in early onset epileptic encephalopathies. Pathogenic variants in the STXBP1 gene are associated with neonatal or infantile onset refractory epilepsy, EEG abnormality, and global developmental retardation. However, some pathogenic STXBP1 variants has been reported with developmental delay, intellectual disability, hypotonia and ataxia without epilepsy. Here, we report a 2 year-old girl patient who was admitted to our clinic with developmental delay, hypotony and dysmorphic features (macrocephaly, flat occiput, wide forehead, cup shaped ears, depressed nasal bridge, hypoplastic nasal wings, short neck and strabismus) without epilepsy. Brain magnetic resonance image (MRI) showed mild hyperintense gliotic signals compatible with mild hypoxic ischemic sequelae. We performed whole exome sequence analysis (WES) and identified a heterozygous splicing mutation (c.1029 +1G>A) in *STXBP1* gene (NM_003165.6). This study reinforces the idea that epilepsy is not a mandatory feature of patients with a STXBP1 mutation.

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P09.132.A Burden of rare variants in *ANK2, AKAP9* and *TSC2* genes supports membrane trafficking and cytoskeletal protein binding as biological processes in patients with severe tinnitus

***Sana Amanat**[1], Alvaro Gallego-Martinez[1], Joseph Sollini[2], Patricia Perez-Carpena[1], Juan M. Espinosa-Sanchez[1,3], Ismael Aran[4], Andres Soto-Varela[5], Angel Batuecas-Caletrio[6], Barbara Canlon[7], Patrick May[8], Christopher R. Cederroth[9,2,7], Jose Antonio Lopez-Escamez[1,3,10]*

[1]GENYO-Centre for Genomics and Oncological Research–Pfizer/University of Granada/Junta de Andalucía, PTS, Granada, Spain, [2]Hearing Sciences, Division of Clinical Neuroscience, School of Medicine, University of Nottingham, Nottingham, United Kingdom, [3]Department of Otolaryngology, Instituto de Investigación Biosanitaria, ibs.Granada, Hospital Universitario Virgen de las Nieves, Granada, Spain, [4]Department of Otolaryngology, Complexo Hospitalario de Pontevedra, Pontevedra, Spain, [5]Division of Otoneurology, Department of Otorhinolaryngology, Complexo Hospitalario Universitario, Santiago de Compostela, Santiago, Spain, [6]Department of Otolaryngology, Hospital Universitario de Salamanca, IBSAL Salamanca Spain, Salamanca, Spain, [7]Laboratory of Experimental Audiology, Department of Physiology and Pharmacology, Karolinska Institute, Stockholm, Sweden, [8]Bioinformatics Core, Luxembourg Centre for System Biomedicine, University of Luxemburg, Esch-sur-Alzette, Luxembourg, [9]National Institute for Health Research (NIHR) Nottingham Biomedical Research Centre, Nottingham University Hospitals NHS Trust, Ropewalk House,

Nottingham, United Kingdom, [10]Department of Surgery, Division of Otolaryngology, University of Granada, Granada, Spain.

Introduction: Severe tinnitus is a heterogeneous condition reported in 1% of the population, showing a significant heritability. It is often associated with hearing loss, hyperacusis or Meniere disease (MD). We aim to identify genes involved in severe tinnitus by using an extreme phenotype (EP) approach.

Materials and Methods: Three independent cohorts with European ancestry (Spanish with MD, Swedish tinnitus and European with generalised epilepsy) were selected to sequence patients with severe tinnitus. We performed a single rare variant analysis (MAF < 0.05) and a gene burden analysis in the SynaptomeDB synaptic genes (N = 1886, MAF < 0.1). Gene ontology (GO) analyses and gene enrichment analyses were performed using GSEA and MsigDB.

Results: We found an enrichment of rare missense variants in 24 synaptic genes including *AKAP9, ANK2* and *TSC2* ($p < 2E^{-04}$).This burden was replicated in the Swedish tinnitus cohort (N = 97) for *ANK2* and in a subset of (N = 34) with severe tinnitus for *ANK2*, *AKAP9* and *TSC2* genes. However, these associations were not significant in the epilepsy cohort without tinnitus (N = 701). *ANK2* coordinates the assembly of several proteins in the axon initial segment (AIS) and drives axonal branching. GO analyses found membrane trafficking and cytoskeletal protein binding in neurons associated with severe tinnitus.

Conclusion: *ANK2, AKAP9* and *TSC2* reveal the main biological processes suggesting that the cytoskeleton organization in AIS could be involved in severe tinnitus.

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P09.133.B Ultrarare structural variation across the genome contributes to severe tinnitus phenotype

Alvaro Gallego-Martinez[1,2], *Sana Amanat*[1,2], *Natalia Trpchevska*[3], *Christopher R Cederroth*[3,4], *Jose Antonio Lopez-Escamez*[1,2,5]

[1]Otology & Neurotology Group CTS 495, Genomic Medicine Area, Centro de Genomica e Investigación Oncológica (GENyO), Pfizer-Universidad de Granada-Junta de Andalucía, Granada, Spain, [2]Department of Otolaryngology, Instituto de Investigación Biosanitaria, ibs.GRANADA, Hospital Universitario Virgen de las Nieves, Granada, Spain, [3]Laboratory of Experimental Audiology, Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden, [4]Division of Clinical Neuroscience, University of Nottingham, Nottingham, United Kingdom, [5]Department of Surgery, Division of Otolaryngology, University of Granada, Granada, Spain.

Introduction: Tinnitus is the most frequent phantom sensation, affecting 70 million individuals in Europe. While prevalence is higher in men, women shows greater psychological burden, suggesting that different coping mechanisms operate between both genders. In this study, we sequenced a Swedish cohort of 97 tinnitus patients aiming to analyse large genomic feature differences between both genders.

Methods: We selected 97 tinnitus Swedish patients according to their reported tinnitus functional index scores through questionaries and performed whole genome sequencing. Variant calling was addressed for different genomic features, separating small indels (SI), Structural Variants (SV) and Copy Number Variants (CNV). We analysed the burden of high constraint variants of each type in both genders comparing their allelic frequencies with Swedish controls from SWEGEN database.

Results: We have found a significant burden of ultrarare SV variants in severe tinnitus patients when compared with Swedish controls ((SV)=*pvalues:* (DUP)=1,2e-08; (DEL)=9,4e-04; (INS)=4,1e-12). However, both genders reported similar burden of damaging variants on the entire genome when considering only structural variants found in high constraint regions.

Conclusion: We report a significant burden of ultrarare structural variation across high constraint regions of the genome for our tinnitus cohort for both genders. This burden increases as we segregate patients with other clinical symptomatology.

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P09.134.C 90% *TSC1/TSC2* mutation detection rate in Tuberous Sclerosis Complex patients without mutation identified in commercial laboratories

Katarzyna Klonowska[1], *Joannes Grevelink*[2], *Krinio Giannikou*[1], *Magdalena Tyburczy*[1], *David Kwiatkowski*[1]

[1]Brigham and Women's Hospital/Harvard Medical School, Boston, MA, USA, [2]Boston Dermatology and Laser Center, Massachusetts General Hospital, Boston, MA, USA.

Introduction: Tuberous sclerosis complex (TSC) is a genetic disorder due to *TSC1*/*TSC2* mutations, characterized by hamartomas involving multiple organs. Our past studies have shown that mosaicism is common in TSC patients who had 'no mutation identified' (NMI) by conventional testing.

Materials and Methods: We used Massively Parallel Sequencing (MPS) for analysis of 144 samples [normal tissues/fluids and TSC tumors (skin: angiofibromas, ungual fibromas, shagreen patch; kidney: angiomyolipomas)] from 30 NMI TSC patients (median age: 33). Mosaic mutations were validated by our new MPS strategy utilizing Unique Molecular Identifier (UMI) based error suppression [sensitivity:0.02% variant allele frequency (VAF)]. Combining these results with previous analysis of 44 mosaic TSC patients, we performed genotype-phenotype correlations.

Results: *TSC1*/*TSC2* mutations were identified in 27 of 30 patients (90%) [21(78%) in *TSC2*; 6(22%) in *TSC1*]; 25 patients had mosaicism [blood VAF:0-19%, median:2.8%]. We identified 7 novel *TSC1*/*TSC2* mutations, including 6 large mutations/rearrangements and a *de novo* deep intronic deletion. We also identified two unique sporadic *TSC2* mutations in each of an angiomyolipoma and a facial angiofibroma, in a patient with minimal TSC clinical features. The mosaic VAF was significantly higher in *TSC1* vs. *TSC2* (median VAF in facial angiofibroma: *TSC1*-6.4%, *TSC2*-4.2%, p = 0.02), although the number of clinical features was similar.

Conclusions: We provide new strategy for very sensitive *TSC1*/*TSC2* mosaic mutation detection and define the spectrum of mosaic mutations and associated clinical features in greater detail than reported previously.

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P09.135.D Tyrosine hydroxylase deficiency associated with dopa-responsive dystonia in a Bulgarian family

Maya Atanasoska[1,2], *Radoslava Vazharova*[1,3], *Lubomir Balabanski*[1], *Slavyana Yaneva-Staykova*[1], *Draga Toncheva*[1,4]

[1]*Gynecology and assisted reproduction hospital "Malinov MD", Sofia, Bulgaria,* [2]*Sofia University St Kliment Ohridski, Faculty of Biology, Department of Genetics, Sofia, Bulgaria,* [3]*Sofia University St Kliment Ohridski, Faculty of Medicine, Department of Biology, Medical genetics and Microbiology, Sofia, Bulgaria,* [4]*Medical University of Sofia, Department of Medical Genetics, Sofia, Bulgaria.*

Introduction: The deficiency of tyrosine hydroxylase leads to autosomal recessive L-DOPA-responsive infantile Parkinsonism and susceptibility to adult-onset L-DOPA-responsive dystonia due to striatal dopamine shortage. Here, we present a family with an affected 1.5-year-old infant, second-born of nonconsanguineous parents, with symptoms of progressive hypokinesia, hypotonia, reduced facial mimicry, diurnal periods of lethargy and irritability, sporadic dystonic movements, developmental delay, and seizures.

Materials and Methods: Genomic DNA from the proband and his family members (mother, father, and a 6-year-old sister) was extracted. Whole exome sequencing was performed for the patient followed by targeted sequencing of the *TH* gene for his family, using an Illumina MiSeq platform.

Results: We identified two heterozygous pathogenic variants in the *TH* gene: c.605G>A(p.Arg202His) and c.614T>C(p.Leu205Pro), associated with an autosomal recessive form of L-DOPA-responsive infantile Parkinsonism. The targeted sequencing confirmed the inheritance of the variants, from the mother (*TH*: c.605G>A) and the father (*TH*:c.614T>C). The sister is a heterozygous carrier of the *TH*:c.614T>C.

Conclusion: DOPA-responsive dystonias are a group of hereditary neurometabolic disorders. The symptoms of the proband correspond to the severe autosomal recessive Segawa syndrome with dystonia onset in the early neonatal period. Beginning treatment, he developed L-dopa hypersensitivity and adverse side-effects. The rest of the family members were non-symptomatic at the time of examination. Studies in a heterozygous knock-in mice-model have shown 60-80% striatal dopamine reduction as a result of the dominant-negative effect of mutant alleles. The outcome is gene dosage depended and individuals with heterozygous *TH* pathogenic variants are expected to be susceptible to adult-onset DRD.

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P09.136.A Diagnostic exome and genome sequencing in complex patients of the Spanish Undiagnosed Rare Diseases Program (SpainUDP)

Beatriz Martinez-Delgado[1,2], *Estrella Lopez*[1], *Sara Monzón*[3], *Isabel Cuesta*[3], *Beatriz Baladrón*[1], *Jose Ignacio Alvarado*[1], *Gema Gomez-Mariano*[1], *Marina Gutierrez*[1], *Julián Lara*[4], *Rosario Cazorla*[4], *Gema Iglesias*[4], *Enriqueta Román*[4], *Purificación Ros*[4], *Pablo Tutor*[4], *Susana Mellor*[4], *Maria Jose Cabrejas*[4], *Angel Zaballos*[5], *Francisco Javier Alonso*[1,2], *Eva Bermejo*[1], *Manuel Posada*[1]

[1]*Instituto de Investigación de Enfermedades Raras, IIER. Instituto de Salud Carlos III, Majadahonda, Madrid, Spain,* [2]*CIBER de Enfermedades Raras (CIBERER), Madrid, Spain,* [3]*Bioinformatics Unit. Instituto de Salud Carlos III, Majadahonda, Madrid, Spain,* [4]*Hospital Puerta de Hierro, Majadahonda, Madrid, Spain,* [5]*Genomics Unit. Instituto de Salud Carlos III, Majadahonda, Madrid, Spain.*

Introduction: The Undiagnosed Rare Diseases Program, SpainUDP, (http://spainudp.isciii.es/) is an institutional Program which has the aim of finding a diagnosis for people with unsolved rare diseases. For this purpose, genomic analysis is applied together with deep phenotyping in a multidisciplinary approach involving clinicians, geneticists, bioinformaticians and researchers.

Materials and Methods: Phenotips was used for an accurate and standardized description of phenotypes (through HPO, Human Phenotype Ontology). Whole Exome Sequencing (WES), and more recently Whole Genome (WGS), were analyzed in Trios. In addition, Transcriptome analysis (RNASeq) was also initiated in 22 cases.

Results: At this moment, genomic analysis through WES was performed in 140 patients. Significantly, we have resolved favorably, establishing the diagnosis of the disease in 43% of the cases, the vast majority of them with pediatric neurological rare syndromes. In 78% of diagnosed cases, the casual variant corresponded to a *de novo* mutation. Additionally, in 11 cases, variants in interesting candidate genes were found which should be further explored for functional validation. This data is being shared through Matchmaker exchange in order to find similar patients, and collaborations were established in 9 cases. Moreover, 13 cases with negative exome results are being analyzed by WGS and/or RNASeq.

Conclusions: WES analysis has allowed us to get a diagnosis in a significant proportion of cases and revealed some recurrent causal genes in our series. WGS and RNASeq analysis is expected to increase the diagnostic rate. Collaborative efforts are important to establish new candidate genes as causal genes defining new disease entities.

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P09.137.B Van Maldergem syndrome 2; An extremely rare case

Deniz Esin, *Fahrettin Duymus, Busra Goksel Tulgar, Ebru Marzioglu Ozdemir, Tulin Cora*

Selcuk University, Konya, Turkey.

Van Maldergem syndrome 2 is an autosomal recessive disease characterized by intellectual disability, dysmorphic craniofacial features, auditory malformations resulting in hearing loss, and limb malformations. This syndrome is caused by mutations in the FAT4 gene. The FAT4 gene encodes a protein that is a member of protocadherins. In this case, we aimed to share the diagnosis process of a 4-year-old boy with Van Maldergem syndrome 2, which is extremely rare.A four-year-old male patient was referred to our clinic with complaints of dysmorphic facial features, poor growth and feeding, and neuromotor retardation. Dysmorphic findings of the patient included microcephaly, bitemporal narrowing, high palate, dental malocclusion, maxillary hypoplasia, and a prominent auricle. The patient also had bilateral hearing loss since birth and spasticity and cryptorchidism. Cranial MR findings of the patient were diffuse polymicrogyria, periventricular calcification, subcortical subependymal heterotopia, pontine hypoplasia, and large cisterna magna. The patient had a history of gastrostomy due to feeding difficulty. Her parents were healthy and have no consanguinity. Whole exome sequence analysis was performed on the patient. FAT4:c.6788 C>T and FAT4:c.3055 C>A heterozygous mutation was detected. As we know to date these

two mutations and the coexistence of these two heterozygous mutations were not found in the literatüre review. In this case report, we emphasize that a novel compound heterozygous FAT4 mutation may cause Van Maldergem syndrome 2 and we aim to contribute to the literature.

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P09.138.C Bi-allelic variants in HOPS subunit VPS41 cause cerebellar ataxia and point to differential lysosomal dysregulation in brain cell types

Leslie E. Sanderson[1], *Kristina Lanko*[1], *Maysoon Alsagob*[2], *Rawan Almass*[3], *Nada Al-Ahmady*[3], *Maryam Najafi*[4,5], *Mohammad Al-Muhaizea*[6], *Hamad Alzaidan*[7], *Hesham Aldhalaan*[6], *Elena Perenthaler*[1], *Herma C. van der Linde*[1], *Anita Nikoncuk*[1], *Nikolas Kühn*[1], *Dinu Antony*[5], *Tarek Owaidah*[8], *Salmo Raskin*[9], *Luana Vieira*[10], *Romulo Mombach*[11], *Najmeh Ahangari*[12], *Taina Silveira*[13], *Najim Ameziane*[13], *Arndt Rolfs*[14,13], *Aljohara Alharbi*[8], *Raghda Sabbagh*[8], *Khalid AlAhmadi*[6], *Bashayer Alawam*[7], *Hazem Ghebeh*[15], *Aljouhra Alhargan*[3], *Anoud Albader*[3], *Faisal Binhumaid*[3], *Faten Almutairi*[3], *Ali Al-Odaib*[3], *Durdane Aksoy*[16], *Nazli A. Basak*[17], *Robin Palvadeau*[17], *Jill Rosenfeld*[18], *Daniah Trabzuni*[19], *Ehsan Karimiani*[20], *Brian Meyer*[3], *Bedri Karakac*[15], *Futwan Al-Mohanna*[21], *Stefan Arold*[22], *Dilek Colak*[23], *Reza Maroofian*[20], *Henry Houlden*[24], *Aida Bertoli-Avella*[13], *Miriam Schmidts*[4,5], *Tahsin S. Barakat*[1], *Tjakko van Ham*[1], *Namik Kaya*[3]

[1]*Erasmus University Medical Center, Dept of Clinical Genetics, Rotterdam, Netherlands,* [2]*King Faisal Specialist Hospital & Research Center (KFSHRC), Dept. of Genetics, Riyadh, Saudi Arabia,* [3]*KFSHRC, Dept. of Genetics, Riyadh, Saudi Arabia,* [4]*Genome Research Division, Human Genetics Dept., Radboud University Medical Center, Nijmegen, Netherlands,* [5]*University Hospital Freiburg, Center for Pediatrics and Adolescent Medicine, Freiburg, Germany,* [6]*KFSHRC, Dept. of Neurosciences, Riyadh, Saudi Arabia,* [7]*KFSHRC, Dept. of Medical Genetics, Riyadh, Saudi Arabia,* [8]*KFSHRC, Dept. of Pathology and Laboratory Medicine, Riyadh, Saudi Arabia,* [9]*Genetika-Centro de Aconselhamento e Laboratório de Genética, Curitiba, Curitiba, Brazil,* [10]*Universidade da Região de Joinville, Programa de Pós- Graduação em Saúde e Meio Ambiente, Santa Catarina, Brazil,* [11]*Prefeitura de Joinville, Núcleo de Assistência Integral ao Paciente Especial, Santa Catarina, Brazil,* [12]*Mashhad University of Medical Sciences, Dept. of Medical Genetics and Molecular Medicine, Mashad, Iran, Islamic Republic of,* [13]*CENTOGENE AG, Rostock, Germany,* [14]*Albrecht-Kossel-Institut for Neurodegeneration, University of Rostock, Rostock, Germany,* [15]*KFSHRC, Dept. of Molecular Oncology, Riyadh, Saudi Arabia,* [16]*Gaziosmanpasa University, Dept. of Neurology, Tokat, Turkey,* [17]*Koç University, Istanbul, Turkey,* [18]*Baylor College of Medicine, Dept. of Molecular and Human Genetics, Houston, TX, USA,* [19]*UCL Institute of Neurology, Dept. of Molecular Neuroscience, London, United Kingdom,* [20]*Genetics Research Centre, Molecular and Clinical Sciences Institute, St George's University of London, London, United Kingdom,* [21]*KFSHRC, Dept. of Cell Biology, Riyadh, Saudi Arabia,* [22]*King Abdullah University of Science and Technology, Computational Bioscience Research Center, Thuwal, Saudi Arabia,* [23]*KFSHRC, Dept. of Biostatistics, Epidemiology and Scientific Computing, Riyadh, Saudi Arabia,* [24]*UCL Institute of Neurology, Dept. of Neuromuscular Diseases, London, United Kingdom.*

Introduction: Membrane trafficking is an essential process in eukaryotic cells responsible for protein transport and processing. Deficiencies in vacuolar protein sorting (VPS) proteins, key regulators of this process, are linked to human disease. VPS proteins function as part of tethering complexes, including the homotypic fusion and vacuole protein sorting (HOPS) complex. While the HOPS-specific subunit VPS41 has been reported to promote viability of dopaminergic neurons in Parkinson's disease, it has not yet been linked to human disease.

Materials and Methods: Whole exome sequencing was performed on nine affected individuals, comprising five unrelated families. *In silico* modeling was performed on suspected causative variants. The consequences of *VPS41* disruption were assessed in vitro using human embryonic stem cells and in vivo using zebrafish.

Results: Affected individuals presented with progressive neurodevelopmental disorder consisting of cognitive impairment, cerebellar atrophy/hypoplasia, nystagmus, and motor dysfunction with ataxia and dystonia. Whole exome sequencing revealed that each individual carried one of four homozygous missense variants in VPS41. In vivo imaging in a genetic zebrafish model indicated lysosomal dysregulation throughout the brain, including significant abnormalities in progenitor cells, microglia, and cerebellar function. In vitro analyses in VPS41 knock-out stem cells confirmed effects of the identified variants on VPS41 expression and function, supporting *in silico* predictions.

Conclusions: Bi-allelic variants in *VPS41* result in lysosomal dysregulation that impacts multiple brain cell types, affects cerebellar function, and contributes to neurodevelopmental disease in humans. Thus, screening for variants in *VPS41* and other HOPS subunits should be considered in cases of unsolved neurodevelopmental disorder.

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P09.139.D Novel *WDR45* frameshift variant detected by whole exome sequencing in beta-propeller protein-associated neurodegeneration disease

Seda Susgun[1,2,3], *Mert Demirel*[4], *Gul Yalcin Cakmakli*[4], *Bulent Elibol*[5], *Sibel Ugur Iseri*[1], *Zuhal Yapici*[6]

[1]*Department of Genetics, Aziz Sancar Institute of Experimental Medicine, Istanbul University, Istanbul, Turkey,* [2]*Graduate School of Health Sciences, Istanbul University, Istanbul, Turkey,* [3]*Department of Medical Biology, Faculty of Medicine, Bezmialem Vakif University, Istanbul, Turkey,* [4]*Department of Neurology, Faculty of Medicine, Hacettepe University, Ankara, Turkey,* [5]*Department of Neurology, Institute of Neurological Sciences and Psychiatry, Hacettepe University School of Medicine, Ankara, Turkey,* [6]*Department of Neurology, Faculty of Medicine, Istanbul University, Istanbul, Turkey.*

Beta-propeller protein-associated neurodegeneration (BPAN) is an X-linked dominant subtype of neurodegeneration with brain iron accumulation (NBIA). It is associated with pathogenic variations in

WDR45 almost exclusively in females due to probable male lethality. Somatic mosaicism has been reported for males diagnosed with BPAN. *WDR45* encodes WD repeat domain 45 and has a main role in autophagy, which is a highly conserved and essential cellular homeostatic process. Clinical features of BPAN include early-onset seizures, developmental delay, intellectual disability, delayed speech, and motor dysfunction. In this study, we have performed whole-exome sequencing (WES) in a male with a potential clinical diagnosis of BPAN at the age of 37. This effort followed by Sanger-based validation and segregation analysis has led us identify a frameshift variant in low level mosaic state in the DNA obtained from the peripheral blood of the affected male. Our next step will be investigating somatic mosaicism using different tissue samples from proband. This work has been supported by the grants of TUBA GEBIP 2019 program.

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P09.140.A Complex cases with Autism Spectrum Disorder (ASD), developmental delay, hyperactivity and sleep disturbance explained by oligogenic mechanisms

Maria Cerminara[1], *Martina Servetti*[1,2], *Margherita Squillario*[2], *Livia Pisciotta*[1,3], *Giovanni Spirito*[4,5], *Maria Teresa Divizia*[2], *Margherita Lerone*[2], *Elisa De Grandis*[1,6], *Silvia Boeri*[6], *Lino Nobili*[1,6], *Diego Vozzi*[5], *Remo Sanges*[4,5], *Federico Zara*[1,2], *Stefano Gustincich*[5], *Aldamaria Puliti*[1,2]

[1]*Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health (DiNOGMI), University of Genova, Genova, Italy,* [2]*Medical Genetics Unit, IRCCS Istituto Giannina Gaslini, Genova, Italy,* [3]*Child Neuropsychiatry Unit, ASST Fatebenefratelli Sacco, Milano, Italy,* [4]*Area of Neuroscience, Scuola Internazionale Superiore di Studi Avanzati (SISSA), Trieste, Italy,* [5]*Department of Neuroscience and Brain Technologies, Istituto Italiano di Tecnologia (IIT), Genova, Italy,* [6]*Child Neuropsychiatry Unit, IRCCS Istituto Giannina Gaslini, Genova, Italy.*

Introduction: Genetic diagnosis of complex ASD cases is often difficult. Emerging evidence suggests that various genetic components can account for these cases according to an oligogenic model. After the observation of a patient with deleterious variants in multiple genes (FrontGenet, https://doi.org/10.3389/fgene.2021.625564), we extended the search for possible oligogenic mechanisms to a cohort of 30 ASD trio families.

Methods: Whole exome sequencing was performed and potentially deleterious variants prioritized by custom filtering strategies including the use of ORVAL (Oligogenic Resource for Variant Analysis Platform) and enrichment analysis of candidate genes with GeneCodis4.

Results: Two cases showed possible deleterious rare variants, each in 3 different genes. A male patient was carrying 2 maternally inherited variants, one hemizygous in *BCOR* and one heterozygous in *MYO9B*, genes associated to cognitive and behaviour impairment. A third heterozygous paternally inherited variant affected *HTR1E*, a serotonin receptor hypothesized to play a role in autism-like behaviour and sleep disturbance. The second case, another male patient, had 2 maternally inherited variants, one hemizygous in *ZC4H2*, associated to developmental delay, and one heterozygous in *ALDH5A1*, associated to behaviour and sleep impairment. A further paternally inherited variant affected *CPLX3*, involved in neurotransmitter release, hypothesized to be implicated in neurodevelopmental delay. Implicated genes revealed enrichment in ASD-associated biological processes and pathways. As our previously described patient, these two patients presented complex ASD phenotype.

Conclusions: About 10% of patients from our ASD cohort showed rare deleterious variants in multiple genes that seem to fully explain their complex phenotype.

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P09.141.B Whole genome sequencing in neurodegenerative diseases: novel variants using different bioinformatics tools

Roberta Croce[1], ***Lucia Corrado***[1], *Nadia Barizzone*[1], *Alice Di Pierro*[1], *Loredana Marialuisa Genovese*[2], *Filippo Geraci*[2], *Eleonora Mangano*[3], *Romina D'Aurizio*[2], *Roberta Bordoni*[3], *Davide Corà*[1], *Francesco Favero*[1], *Miriam Zuccalà*[1], *Cristoforo Comi*[1], *Fabiola De Marchi*[4], *Luca Magistrelli*[4], *Giovanni Manzini*[2,5], *Project MinE ALS sequencing consortium, Gianluca De Bellis*[3], *Alfredo Brusco*[6], *Marco Severgnini*[3], *Marco Pellegrini*[2], *Letizia Mazzini*[4], *Sandra D'Alfonso*[1]

[1]*University of Eastern Piedmont UPO, Novara, Italy,* [2]*Institute of Informatics and Telematics of CNR, Pisa, Italy,* [3]*National Research Council of Italy, Institute for Biomedical Technologies, Segrate (Milano), Italy,* [4]*ALS Center AOU Maggiore della Carità, Novara, Italy,* [5]*University of eastern Piedmont, UPO, Alessandria, Italy,* [6]*University of Torino, Torino, Italy.*

We explored missing heritability in 140 patients affected by three different Neurodegenerative disorders (NDDs). We performed Whole Genome Sequencing after excluding pathogenic variants in the main causative genes and investigated three classes of potentially pathogenic variants: a) Coding/non-coding SNV/Indels in a panel of 696 genes involved in NDDs. Using standard annotation, we identified pathogenic/likely pathogenic variants in genes causative of rare forms of each disease (N = 15) and in gene causing a NDD different from patient clinical presentation (N = 16). SpliceAI, a deep learning tool predicting an effect on splicing mechanism identified 48 variants with a possible splicing impact. We performed in vitro studies for 9 variants and confirmed a role in splicing alteration for 6 of them. b) Genome-wide structural variants. Using CNVkit, we identified a 15q25 deletion in a PD patient. Similar deletions have been associated with mild intellectual disability and dysmorphisms but never reported in PD cases. c) Genome wide Tandem Repeat (TR). Using literature and novel tools, we identified four novel loci in ALS cohort with a possible TR expansion and performed a replication of the results in larger independent cohorts from Italy and International MinE project. For 3 of them (*FRA10AC1*, *RFC1*, *HK1*) the result was not replicated. For *ITFG2*, preliminary data are promising since the TR was observed only in patients and in none controls. In conclusion, using WGS data we were able to find missed pathogenetic variants in genes associated with different NDDs, reinforcing the idea of a shared genetic cause among these diseases.

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P09.142.C Genetic variation spectrum of *ATP7B* in a cohort of 113 patients with Wilson disease

João Parente Freixo[1], *Ana Lopes*[1], *Rita Bastos-Ferreira*[1], *Henedina Antunes*[2,3,4], *Ermelinda Santos Silva*[5], *Jorge Sequeiros*[1], *Marina Magalhães*[6], *Jorge Oliveira*[1]

[1]*CGPP - Centro de Genética Preditiva e Preventiva, IBMC - Instituto de Biologia Molecular e Celular, i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal,* [2]*Pediatric Gastroenterology Hepatology and Nutrition Unit, Hospital de Braga, Braga, Portugal,* [3]*2CA – Clinical Academic Center, Braga, Portugal,* [4]*ICVS - Life and Health Sciences Research Institute, School of Medicine, University of Minho, Braga, Portugal,* [5]*Unidade de Gastroenterologia Pediátrica, Serviço de Pediatria, Centro Materno-Infantil do Norte, Centro Hospitalar Universitário do Porto, Porto, Portugal,* [6]*Serviço de Neurologia, Centro Hospitalar Universitário do Porto, Porto, Portugal.*

Wilson disease (WD) is an autosomal recessive disorder of the copper metabolism, caused by diallelic pathogenic variants in the copper-transporting gene, *ATP7B*. WD usually presents with hepatic, neurologic, and/or psychiatric disturbances. Molecular genetic testing is critical for a timely-adequate diagnosis and treatment, to prevent lifelong disabilities. This work aimed at expanding the mutational spectrum of disease-related variants in *ATP7B*, in a large cohort of WD patients. Since 2004, a total of 301 patients were genotyped at CGPP, for confirmation or exclusion of WD. In the vast majority of patients, *ATP7B* gene was analysed by Sanger sequencing and, in patients heterozygous for one disease-causing variant (n = 20), MLPA was also performed. Variants were classified according to the ACMG guidelines. WD was genetically confirmed in 113 patients (99 families): 32 are homozygotes and 81 compound heterozygotes for pathogenic or likely-pathogenic variants. A total of 34 distinct variants (including *4* novel) and 64 different genotypes were determined. The three most common disease-causing variants were found in 75.2% of the cases, among whom 18 were homozygotes; NM_000053.3:c.3402del was the most frequent, being present in homozygosity in 6 and in heterozygosity in 19 patients. This data expands the mutational spectrum of WD causing variants and contributes to the continuously demanding effort of interpreting variants causing WD. Interestingly, the c.3402del variant has also been reported as the most frequent in WD cohorts from Venezuela and Brazil. This contrasts with other European or Asian cohorts, where p. His1069Gln or p.Arg778Leu, respectively, seem to be the most prevalent WD-causing alleles.

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P10 Neuromuscular Disorders

P10.001.A Objective evaluation of clinical actionnability for genes involved in myopathies: 51 promising genes

Maude Vecten[1,2], ***Emmanuelle Pion***[3], *Raul Juntas Morales*[4], *Damien Sternberg*[5], *John Rendu*[6], *Tania Stojkovic*[7], *Cécile Acquaviva-Bourdain*[8], *Corrine Métay*[9], *Isabelle Richard*[10], *Laurent Villard*[2], *Mathieu Cérino*[11,2], *Mathieu Milh*[12,2], *Sevtlana Gorokhova*[13,2], *Nicolas Levy*[13,2], *Xenia Martin*[6], *Gisele Bonne*[7], *Valérie Biancalana*[14], *François Petit*[15], *Aurélien Perrin*[16], *Pascal Laforet*[7], *Marc Bartoli*[2], *Mireille Cossée*[17], *Martin Krahn*[13,2]

[1]*Département de Génétique, Hôpital Bichat – Claude-Bernard, APHP, Paris, France,* [2]*Aix-Marseille Université, INSERM, Marseille Medical Genetics, U1251, Marseille, France,* [3]*Filnemus, Laboratoire de Génétique Moléculaire, CHU de Montpellier, Montpellier, France,* [4]*Département de Neurologie, Hôpital Gui de Chauliac, CHU de Montpellier, Montpellier, France,* [5]*Service de Biochimie Métabolique, Hôpitaux Universitaires Pitié Salpétière, APHP, Paris, France,* [6]*Laboratoire de Génétique Moléculaire, CHU de Grenoble et des Alpes, Grenoble, France,* [7]*Institut de Myologie, Hôpital Pitié-Salpêtrière, APHP, Paris, France,* [8]*Service Biochimie et Biologie Moléculaire Grand Est - UM Maladies Héréditaires du Métabolisme, HCL, Lyon, France,* [9]*UF Cardiogénétique et Myogénétique et Cellulaire, Centre de Génétique moléculaire et chromosomique, Hôpitaux Universitaire Pitié-salpétière, APHP, Paris, France,* [10]*Centre de Recherche Généthon Institut des Biothérapies Généthon, Paris, France,* [11]*Laboratoire de Biochimie, Hôpital de la Conception, APHM, Marseille, France,* [12]*Service de Neuropédiatrie, Hôpital Timone Enfants, APHM, Marseille, France,* [13]*Département de Génétique Médicale, Hôpital Timone Enfants, APHM, Marseille, France,* [14]*Laboratoire de diagnostic génétique CHU de Strasbourg, Strasbourg, France,* [15]*Laboratoire de génétique moléculaire Service de biochimie et hormonologie Hôpital Antoine Béclère, APHP, Clamart, France,* [16]*PhyMedExp, Université de Montpellier, INSERM, CNRS, Laboratoire de Génétique Moléculaire, CHU de Montpellier, Montpellier, France,* [17]*Laboratoire de Génétique Moléculaire, CHU de Montpellier, Montpellier, France.*

Introduction: The implementation of high-throughput diagnostic sequencing has led to the generation of large amounts of mutational data, making their interpretation more complex and responsible for long turnaround times. It has been important to prioritize certain analyses, particularly those of "actionable" genes in diagnostic situations, involving specific treatment and/or management. In our project, we carried out an objective assessment of the clinical actionability of genes involved in myopathies, for which only few data obtained methodologically exist to date.

Materials and methods: Using the ClinGen Actionability criteria, we scored the clinical actionability of all 200 associated genes for myopathies published by FILNEMUS for the "National French consensus on gene Lists for the diagnosis of myopathies using next generation sequencing".

Results: We objectified that 51 associated geènes for myopathies were actionable with currently available data. Among In theise 52 genes only 14 had beenve scored to date by ClinGen.

Conclusion: The data obtained through these methodological tools are an important resource for strategic choices in diagnostic approaches and the management of genetic myopathies. The clinical actionability of genes has to be considered as an evolving concept, in relation to progresses in therapeutic approaches.

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P10.002.B *ACTN3* rs1815739 polymorphism is not associated with sports injuries in Slovenian female football players

Inge Sotlar[1], *Tisa Podkrajšek*[1], *Tina Levstek*[1], *Katja Goričar*[1], *Vita Dolžan*[1], *Katarina Trebušak Podkrajšek*[1,2]

[1]*Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia,* [2]*Clinical Institute for Special Laboratory Diagnostics, University Children's Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia.*

Introduction: Alpha-actinin-3 is a protein expressed in fast-twitch (type II) muscle fibres. Genetic variant NM_001104.4:c.1729C>T (p. R577X) (rs1815739) in *ACTN3* introduces premature termination codon. In homozygous form, it reduces strength, muscle mass and

fast-twitch fibre diameter. In athletes (including professional football players), it was associated with a higher risk of sports injuries and longer exercise recovery. We aimed to evaluate the association of the p.R577X with sports injuries in Slovenian female football players.

Materials and Methods: The study group included 43 female football players older than 13 years and actively training football more than 4 years. Data collected included player's position and frequency of sports injuries. *ACTN3* p.R577X genotyping was performed on saliva DNA with PCR and *Dde*I restriction analysis with subsequent Sanger sequencing confirmation, if necessary.

Results: The median (range) age of the group was 16 (13–28) years; they were actively training football for a median of 7 (4–18) years. p.R577X in homozygous or heterozygous form was present in 58.1% of the players, while 41.9% had normal genotype. 53.7% had a history of at least one sports injury, namely sprains, bone fractures, muscle, or other injuries. However, no statistically significant association was found between genotype and the presence of injuries ($p = 0.309$), nor between the player position and the presence of injuries or genotype and player position ($p = 0.598$ and $p = 0.830$, respectively).

Conclusion: No association was found between *ACTN3* p.R577X genotypes and sports injuries or player position in our study, although further studies with larger cohort are needed to verify the results.

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P10.003.C Utilising gold-standard PCR genotypes to accurately infer repeat expansions from whole-genome sequence data

Mark A. Doherty, ***Laura O'Briain****, Jennifer Hengeveld, Éanna MacDomhnaill, Orla Hardiman, Russell L. McLaughlin*

Trinity College Dublin, Dublin 2, Ireland.

Background: We present a pilot study to ultimately develop a pipeline utilising in silico tools to identify and accurately infer the lengths of known and novel pathogenic repeat expansions (REs) in amyotrophic lateral sclerosis (ALS) from whole-genome sequencing (WGS) data. This pipeline will benefit from having both WGS data and gold-standard PCR genotypes of multiple REs. Despite being highly heritable, pathogenic variants are only identified in approximately 15% of ALS cases. The most common known cause of ALS is a hexanucleotide RE in *C9orf72*. Other intermediate REs in *NIPA1*, *ATXN1* and *ATXN2* are known ALS risk factors. In this preliminary study we evaluate the ability of in silico tools to assess the lengths of *ATXN2* CAG REs.

Methods: 221 cases and 117 controls are included in this initial study. Both WGS and gold-standard *ATXN2* CAG PCR genotypes are available for all individuals. The WGS data were interrogated using a suite of bioinformatic tools.

RESULTS: Several tools including hipSTR, lobSTR, tredparse and ExpansionHunter accurately measure *ATXN2* CAG REs (route-mean-square deviations (RMSDs): 1.2). The worst performing software was gangSTR (RMSD: 12.8), which falsely identified CAG repeats elsewhere in the genome.

Discussion: This project utilises high-quality WGS data and gold-standard PCR data. We accurately infer the lengths of *ATXN2* CAG repeats using in silico tools; however, certain gene and repeat specific properties can negatively impact results. We now aim to expand this study to a larger gene panel for which accurate PCR genotypes are available and to a larger international sample size.

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P10.004.D The pleiotropy of neurodegenerative repeat expansions in ALS

Jennifer C. Hengeveld[1]*, Alice Vajda*[2]*, Mark Heverin*[2]*, Orla Hardiman*[2]*, Russell L. McLaughlin*[1]

[1]*Smurfit Institute of Genetics, Trinity College Dublin, Dublin, Ireland,* [2]*Academic Unit of Neurology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland.*

Introduction: Repeat expansions (REs) underlie more than 40 diseases, most of them affecting the nervous system. The most common neurodegenerative repeat expansions (NDREs) diseases are Huntington's Disease (HD), Spinocerebellar Ataxias (SCA), Frontotemporal Dementia (FTD) and Amyotrophic Lateral Sclerosis (ALS). ALS is a fatal neurodegenerative disorder which causes the death of neurons controlling voluntary muscles. ALS has no cure, and its underlying cause is mostly unknown, although a strong genetic component is known to play a role. Several REs are pleiotropic; for example, GGGCC RE in *C9orf72* is associated with FTD/ALS and CAG RE in *ATXN2* causes SCA2/ALS. Previous studies on *ATXN2* showed that harbouring intermediate-length repeat expansions are significantly associated with the risk of ALS. Therefore, pleiotropy might be common in ALS. This study aims to genotype 34 neurodegenerative genes that harbour REs, in a cohort of 1000 controls and 1000 patients from the Irish ALS bank to assess the association between expanded genotypes and ALS.

Materials and Methods: The length measurement of each NDRE gene and its possible repeat expansions was done by PCR, Repeat Primed-PCR (RP-PCR), agarose gel electrophoresis and fragment length capillary electrophoresis.

Results: In an Irish population, ALS might be driven by multiple intermediate-length repeat expansion in likely 8 NDREs genes: *ATXN2*, *DIP2B*, *FRA11AC1*, *FRA11A*, *NUTM2B-AS1*, *PABN1*, *TK2-BEAN* and *ZNF713*.

Conclusions: ALS is a very complex disease that might be caused by pleiotropy of multiple REs and multiple factors.Funding: Science Foundation Ireland (17/CDA/4737)

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P10.006.B Dissecting the sex-dependent genetic architecture of amyotrophic lateral sclerosis

Ross P. Byrne[1]*, Wouter van Rheenen*[2]*, Jan H. Veldink*[2]*, Russell L. McLaughlin*[1]

[1]*Smurfit institute of genetics, Trinity College Dublin, Dublin, Ireland,* [2]*Department of Neurology, Brain Centre Rudolf Magnus, University Medical Center Utrecht, Utrecht, Netherlands.*

Introduction: Amyotrophic lateral sclerosis (ALS) is a late-onset neurodegenerative disease characterised by progressive loss of upper and lower motor neurons. Sex modifies both disease risk and heritability, with higher incidence in males and higher rates of mother to daughter transmission. However, the extent that sex affects the genetic architecture of ALS is currently understudied. We reanalysed genetic data from a published ALS GWAS ($N = 36{,}052$) to assess sex differences in genetic architecture of the disease.

Methods: We fit sex as an interaction term in a GCTA-GREML model to evaluate its impact on ALS heritability. Next, we ran GWAS on male-specific (N = 18,732) and female-specific (N = 17,322) subsets of the data using linear mixed models. Sex specific loci were identified in males and females by scanning for variants significant at a 5% FDR in one sex, but not even nominally significant in the other (p>0.05). Both global and regional sex-specific heritabilities were estimated using LD-score regression and Heritability Estimation from Summary Statistics (HESS).

Results: We observed significant evidence of gene by sex interactions that account for ~1/3rd of ALS SNP heritability (likelihood-ratio test: p = 0.0087). Female-specific heritability (h2=0.043;SE = 0.01) was substantially higher than male-specific heritability (h2=0.001;SE = 0.01) and regional heritability analysis revealed greater polygenicity in females. Finally, our sex specific scan identified several known (MOBP, C9orf72, SARM1, UNC13A) and novel (PIP5K1B, ATP8A2, PCDH9, RNASE9, OTUD7A, ITPRIPL2, UNK, FBF1) loci harbouring SNPs associated with ALS in only one sex. These loci were enriched for expression in brain tissue consistent with the known aetiology of ALS. **Grants:** SFI 17/CDA/4737

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P10.007.C Bi-allelic loss of *ERGIC1* in relatively mild arthrogryposis

Caterina Marconi[1], *Laure Lemmens*[1], *Frédéric Masclaux*[1], *Francesca Mattioli*[1], *Joel Fluss*[2], *Philippe Extermann*[3], *Purificacion Mendez*[4], *Russia Ha-Vinh Leuchter*[2], *Elisavet Stathaki*[1], *Sacha Laurent*[1], *Anne Vanier*[1], *Konstantinos Varvagiannis*[1], *Michel Guipponi*[1,5], *Frédérique Sloan-Bena*[1,6], *Jean-Louis Blouin*[1,6], *Abramowicz Marc*[1], *Siv Fokstuen*[1,6]

[1]*Genetic Medicine division, Diagnostic Department, Hôpitaux Universitaires de Genève, Geneva, Switzerland,* [2]*Pediatric Specialties division, Department of Women, Children and Adolescents, Hôpitaux Universitaires de Genève, Geneva, Switzerland,* [3]*Dianecho, Geneva, Switzerland,* [4]*Centre Médical Eaux-Vives, Geneva, Switzerland,* [5]*Department of Genetic Medicine and Development, School of Medicine, Uniersity of Geneva, Geneva, Switzerland,* [6]*Department of Genetic Medicine and Development, School of Medicine, University of Geneva, Geneva, Switzerland.*

Arthrogryposis is a descriptive term that defines the presence of multiple joint-contractures. Clinical severity of this phenotype is variable and ranges between mild joint-only to severe multi-organ involvements. So far, more than 400 genes have been reported as causative. Among these, *ERGIC1* is a recently proposed candidate gene that encodes a putative transmembrane protein of the Endoplasmic Reticulum-Golgi interface. Two homozygous missense variants have been reported in patients with relatively mild non-syndromic arthrogryposis. We report on a consanguineous family with two affected siblings presenting relatively mild congenital arthrogryposis of upper and lower limbs, and some facial dysmorphism. Whole genome sequencing revealed a homozygous 22.6Kb deletion encompassing the promoter and first exon of *ERGIC1*. We mapped the breakpoints at nucleotide-level resolution, showing the involvement of two 86% similar *Alu* elements in the rearrangement. RNA studies demonstrated the complete absence of *ERGIC1* expression in the two affected siblings and a nearly 50% decrease in the heterozygous parents. Our data allowed to establish the pathogenic role of *ERGIC1* in congenital arthrogryposis by demonstrating the loss-of-function pathogenic mechanism associated to a relatively mild arthrogryposis phenotype even with the complete absence of *ERGIC1* expression. This contributes to a better comprehension of *ERGIC1* function and to improve genetic counseling of *ERGIC1* mutations, especially in a prenatal setting.

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P10.008.D At birth hypertony and arthrogryposis: expanding the phenotypic spectrum of variants in the Filamin C *(FLNC)* gene

Nikola Čajbiková[1], *Pavel Tesner*[1], *Martin Schwarz*[1], *Veronika Moslerová*[1], *Miroslava Balaščaková*[1], *Lukáš Ryba*[1], *Anna Křepelová*[1], *Lucie Dušková*[2], *Jana Zídková*[2], *Lenka Fajkusová*[2], *Markéta Havlovicová*[1], *Milan Macek, jr*[1]

[1]*Department of Biology and Medical Genetics, Prague, Czech Republic,* [2]*Centre of Molecular Biology and Genetics, University Hospital Brno, Brno, Czech Republic.*

Introduction: The Filamin C gene (*FLNC;* MIM: 102565) is known for its association with various cardiomyopathies and myopathies. Here we report two cases of arthrogryposis multiplex congenita (MIM: 208100), which presented at birth through hypertony, as a new phenotypic presentation of pathogenic variants in *FLNC*.

Materials: Both probands come from non-consanguineous Czech families with a negative family history. The first case is a two-year-old boy, diagnosed at birth with symmetric hypertonic syndrome and arthrogryposis of his shoulders. Cardiological investigation revealed a mild form of hypertrophic cardiomyopathy. The second case is a seven-year-old boy diagnosed after birth also with neck hypertony and limited mobility of his shoulder girdle. His first cardiological examination was performed at the age of 4 years resulting in the diagnosis of atypical cardiomyopathy.

Methods: Massively parallel sequencing (Illumina, USA) was performed in both cases, followed by Sanger DNA sequencing in order to verify detected variant segregation in available first-degree relatives.

Results: A *de novo* heterozygous likely pathogenic (Class 4) variant c.3557C>T p.(Ala1186Val) in *FLNC* was found in both patients, while additional pathogenic variants were not detected.

Conclusions: The phenotype of congenital arthrogryposis associated with *FLNC* was described only recently, in three cases. Our patients have the same pathogenic variant as two of them, suggesting that this might be a recurrent variant. Our observation underscores the importance of the analysis of *FLNC* in patients with at birth hypertony and/or arthrogrypotic clinical features. Supported by MH CZ - DRO, Motol University Hospital, Prague, Czech Republic 00064203

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P10.009.A Mutations of *ATP1A3* in residue 756 cause a new phenotype, case report and literature review

Mateusz Biela[1], *Małgorzata Rydzanicz*[2], *Krystyna Szymańska*[3], *Karolina Pieniawska-Śmiech*[1], *Aleksandra Lewandowicz-Uszyńska*[1], *Joanna Chruszcz*[1], *Leszek Szenborn*[1], *Aleksandra Jakubiak*[1], *Rafał Płoski*[2], *Robert Śmigiel*[1]

[1]Wroclaw Medical University, Wroclaw, Poland, [2]Warsaw Medical University, Warsaw, Poland, [3]Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland.

Heterozygous mutations in the *ATP1A3* gene cause different phenotypes like: alternating hemiplegia of childhood (AHC), rapid-onset dystonia-parkinsonism (RDP), catastrophic infantile epilepsy with microcephaly, and cerebellar ataxia, areflexia, pes cavus, optic atrophy, sensorineural hearing loss (CAPOS) and severe early infantile epileptic encephalopathy. In 2017 a new phenotype was proposed by Yano et al. for patients with mutations in residue 756 - Fever-Induced Paroxysmal Weakness and Encephalopathy (FIPWE), next Sabouraud et al. suggested Relapsing Encephalopathy with Cerebellar Ataxia (RECA) in children with *ATP1A3* mutation. Here we present a case of a boy with 2 episodes of severe hypotonia with depressed deep tendon reflexes and speech disorder, strabismus and ataxia triggered by a febrile infection. In WES analysis performed in rapid mode a *de novo ATP1A3* mutation (R756H) was found. Additionally, we have analyzed 34 (including our) cases with mutation in residue 756 of aminoacid sequence described in literature. In the analyzed group of *ATP1A3* mutation patients, all paroxysmal episodes (59 in total) were triggered by fever. During the paroxysmal episodes the most common symptoms were: hypotonia (82.4%), symptoms including the orofacial area (85.3% i.a. dysarthria, dysphagia, mutism), ataxia (76.5%) and cognitive decline (61.8%). Recovery was usually slow and not always full. Taking into account the overall symptoms and the repeatability of the phenotype, we suggest delineating a separate disease entity and support the acronym FIPWE. Financed from the funds granted by the Ministry of Science and Higher Education in the Regional Initiative of Excellence program for the years 2019-2022, project number 016/RID/2018/19.

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P10.010.B Genetic investigation of a Brown Vialetto Van Laere family from Southern Italy

Benedetta Perrone[1], *Paola Ruffo*[1], *Sebastiano Andò*[1], *Francesca Luisa Conforti*[1], *Vincenzo La Bella*[2]

[1]University of Calabria, Cosenza, Italy, [2]University of Palermo, Sicilia, Italy.

Brown-Vialetto-Van Laere syndrome is a debilitating neurodegenerative disease with an incidence of about 1 in 1,000,000 people in the general population. It is considered a juvenile form of Amyotrophic lateral sclerosis (ALS) characterized by progressive pontobulbar palsy associated with sensorineural deafness. In this report, we present a study of a family in which a young female was affected by BVVL. We performed genetic analysis by Sanger sequencing for riboflavin transporters genes and targeted Next-generation sequencing (NGS) panel, containing all genes currently associated with ALS. Our results showed 5 known variants in *C20orf54* gene both in the proband and in healthy family members (I74M in exon 2 and P267L, T278M, I303V, R266W in exon 3). Among these variants, we focused our attention on the heterozygote mutation R266W, that could have a deleterious consequence for the protein structure perturbing its function. In addition, NGS revealed a new frameshift deletion in exon 19 of the *TBK1* gene (I669Sfs*). This gene plays a key role in the phosphorylation of several protein, promoting autophagy. This ability depends on the CCD2 domain integrity and I669Sfs*, falling within that protein portion, could cause the accumulation of protein aggregates. Our findings indicate that the *C20orf54* mutation R266W alone is not sufficient to trigger BVVL but, together with other specific variants, it could orchestrate a particular genetic spectrum able to induce BVVL. Furthermore, we suggest for the first time that a new variant in *TBK1* gene could exert a crucial action in suppressing autophagy mechanisms, contributing to promote BVVL.

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P10.012.D In-depth characterization of mutations causing axonal recessive peripheral neuropathy with neuromyotonia (NMAN): the structure gives a HINT

Silvia Amor-Barris[1,2], *Tamás Lázár*[3,4], *Kristien Peeters*[1,2], *Shoshana Wodak*[3,4], *Albena Jordanova*[1,2,5]

[1]VIB - Center for Molecular Neurology, Antwerp, Belgium, [2]University of Antwerp, Antwerp, Belgium, [3]VIB - Center for Structural Biology, Brussels, Belgium, [4]Vrije Universiteit Brussel, Brussels, Belgium, [5]Medical University-Sofia, Sofia, Bulgaria.

Introduction: Loss-of-function mutations in *HINT1* were identified to cause axonal recessive peripheral neuropathy with neuromyotonia (NMAN). Patients suffered from motor-greater-than-sensory polyneuropathy with an age of onset mostly within the first decade of life. Moreover, 70% of patients present with the hallmark of neuromyotonia. Currently, 25 NMAN variants have been described, predominantly in sporadic cases and small families, most of them with limited functional evidence of pathogenicity. We systematically characterized all reported variants aiming to dissect the loss-of-function mechanism.

Methods: Each variant was mapped in the crystal structure of HINT1 and *in silico* folding stability predictions were performed. Stability and functionality of the resulting proteins were tested in vivo using HINT1 KO cells and a yeast model deficient for *HNT1* (yeast *HINT1* orthologue).

Results: Mapping of all NMAN-causing variants allowed their classification into three structural clusters: a) catalytic pocket; b) dimer interface; c) β-sheet behind the catalytic pocket. Folding stability predictions pointed towards monomer instability for variants in the β-sheet and dimer instability for variants at the dimer interface. These predictions were confirmed in vivo, and we correlated each structural cluster to different loss-of-function mechanisms: mutations in the catalytic pocket rendered a non-functional protein; mutations at the dimer interface led to unstable proteins; mutations in the β-sheet were highly unstable but retained some enzymatic activity.

Conclusions: We clustered all HINT1 mutations in three different structural groups allowing for a potential patient stratification strategy for future treatments. Our study enables us to predict and validate the pathogenicity of newly identified variants.

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P10.013.A Screening of *SORD* mutations in a CMT cohort expands the clinical spectrum of *SORD*-related neuropathy

Camila Armirola-Ricaurte[1], *Els de Vriendt*[1], *Ayse Candayan*[2], *Ognyan Asenov*[3], *Yesim Parman*[4], *Teodora Chamova*[3], *Ivailo Tournev*[3], *Esra Battaloglu*[2], *Albena Jordanova*[1,5]

[1]VIB-UAntwerp Center of Molecular Neurology, Antwerp, Belgium, [2]Department of Molecular Biology and Genetics, Bogazici University, Istanbul, Turkey, [3]Department of Neurology, University Hospital Alexandrovska, Medical University-Sofia, Sofia, Bulgaria, [4]Department of Neurology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey, [5]Molecular Medicine Center, Department of Medical Chemistry and Biochemistry, Medical University-Sofia, Sofia, Bulgaria.

Introduction: Charcot-Marie-Tooth neuropathies (CMT) are incurable diseases, and collectively, they are the most common genetic disorder affecting peripheral neurons. Mutations in *SORD* have been recently identified as a frequent and potentially treatable cause of autosomal recessive CMT, presenting as axonal or predominantly distal motor neuropathy. In light of this discovery, we aimed to evaluate the impact of *SORD* mutations in a cohort of unsolved individuals with CMT.

Methods: We analyzed 720 unrelated patients, predominantly of South-Eastern European and Turkish ancestry, with sporadic or recessive CMT who remained unsolved after targeted re-sequencing of the most common CMT genes. Sanger sequencing was used to screen all probands for mutations in exon 7 of *SORD*, which harbors *SORD*'s most common pathogenic variant c.757delG (p.Ala253GlnfsTer27). Targeted sequencing of the remaining exons was performed in cases where only one heterozygous pathogenic variant was found in exon 7.

Results: We identified 12 individuals homozygous for the c.757delG mutation. Five of them were diagnosed with axonal CMT, three with demyelinating CMT and one patient with intermediate CMT. Three adult siblings with the c.757delG homozygous variant exhibited phenotypic variability, as only one of them reported symptoms whereas the others were asymptomatic. Interestingly, one Bulgarian patient with axonal CMT also experienced pyramidal and cerebellar symptoms. We identified 10 individuals heterozygous for the c.757delG mutation. One of them carried additionally a variant of unknown significance (c.951T>G, p.Asn317Lys).

Conclusions: This work confirms the relevance of *SORD* as a causal gene for CMT disorders and expands the phenotypic spectrum of *SORD* neuropathy.

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P10.014.B Uniform *Drosophila* models for four CMT-related aminoacyl-tRNA syntheses reveal common signs of toxicity

Laura Morant[1,2], *Maria Luise Petrovic-Erfurth*[1,2], *Albena Jordanova*[1,2]

[1]VIB - Center for Molecular Neurology, Antwerp, Belgium, [2]University of Antwerp, Antwerp, Belgium.

Introduction: Charcot-Marie-Tooth disease (CMT) is characterized by demyelination and/or axonal degeneration of peripheral motor and sensory neurons. Six aminoacyl-tRNA synthetases (aaRS) have been associated to CMT etiology. Interestingly, loss of aminoacylation-activity does not cause CMT, suggesting a toxic gain of function mechanism. We aim to investigate whether there is a common pathomechanism underlying aaRS-associated CMT.

Methods: We generated fly models for four CMT-related aaRS by expressing two pathogenic mutations (one altering and one not affecting the enzymatic activity) using a modified GeneSwitch™ technology. Transgenes were injected into the same landing sites of the fly genome and verified by Sanger sequencing. AaRS protein expression levels were quantified by immunoblotting. General toxicity and locomotor function were evaluated via developmental lethality and negative geotaxis climbing assays.

Results: Strong ubiquitous expression of mutant aaRS induced developmental lethality in all four aaRS Drosophila models. Reducing mutant transgene levels restored fly viability, suggesting that toxicity of aaRS-mutants is dosage dependent. Males flies were more affected than females, suggesting gender specific vulnerability. Ageing flies pan-neuronally expressing mutant aaRS displayed reduced locomotion, mimicking the progressive walking impairment features of CMT patients. Overall, our findings are similar to previously described phenotypes in YARS and GARS fly models, confirming that phenotype expressivity does not correlate with aminoacylation-activity of aaRS.

Conclusion: Expression of CMT-causing mutations caused similar signs of toxicity in four CMT-related aaRS, rendering our models a valid platform for investigating putative shared molecular pathway(s). The knowledge gained might contribute to common treatment strategies for all aaRS-related neuropathies.

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P10.015.C Additive effect of frequent and rare synonymous variants as the cause of altered *NPC1* splicing in twin patients with Niemann-Pick disease type C

Igor Bychkov, *Aleksandra Filatova, Tatiana Proshlyakova, Vyacheslav Tabakov, Galina Baydakova, Alexandra Ilyushkina, Mikhail Skoblov, Ekaterina Zakharova*

Research centre for medical genetics, Moscow, Russian Federation.

Background: Niemann-Pick disease type C (NPC) is an autosomal recessive disorder caused by mutations in either the *NPC1* or *NPC2* genes. Two 55 y.o. twins were suspected for adult form of NPC, based on clinical and biochemical symptoms and were referred for a genetic testing.

Results: NGS analysis of patient`s DNA revealed two rare compound heterozygous variants in *NPC1*: typical loss-of-function c.2196dup (p.Ala732fs*30) mutation and c.2727C>T (p.Cys909=) variant of unknown significance. Analysis of the patients` cDNA showed that c.2727C>T variant causes cryptic donor splice site (DS) activation and 74 b.p. deletion in *NPC1* exon 18. As patients also had frequent c.2793C>T (p.Asn931=) variant in homozygous state, located in wild type DS of the same exon, we hypothesized, whether it could affect the competitive activity of two DSs. Minigene assay demonstrated that the significant decrease of WT transcript isoform and the significant amount of shortened isoform are observed only when both variants are present in cis.

Conclusion: The novel complex allele c.[2727C>T;2793C>T] in *NPC1* is functionally characterized. Minigene assay demonstrated that it is a "leaky" spliceogenic mutation, which leads to approximately 50% reduction of WT transcript isoform. In compound heterozygous state with typical loss-of-function mutation it causes adult onset form of NPC. The results of this study highlight the necessity of analyzing the impact of genomic milieu in cases when identified mutation is spliceogenic, as any additional variant in close proximity could significantly affect the ratio of transcript isoforms and lead to a misconception in genotype-phenotype correlations or even wrong diagnosis.

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P10.016.D Isoform specific variant as a potential cause of distal myopathy

Jean Mezreani[1,2], *Florence Martin*[1], *Sébastien Audet*[1,2], *Jade Charbonneau*[1], *Erik Bareke*[1], *Annie Laplante*[1], *Bernard Brais*[3,4], *Erin O'Ferrall*[3,4], *Jason Karamchandani*[3,4], *Martine Tétreault*[1,2]

[1]*CRCHUM, Montreal, QC, Canada,* [2]*Neurosciences Department, UdeM, Montreal, QC, Canada,* [3]*MUHC, Montreal, QC, Canada,* [4]*Rare Neurological Diseases Research Group, Montreal, QC, Canada.*

Introduction: Despite recent advance in next-generation DNA-sequencing technologies, at least 25% of myopathy patients remain without a genetic diagnosis. In order to increase diagnostic yield, we combined omic technologies to identify novel gene mutations in a cohort of 17 patients with rare undiagnosed myopathies. Among those, we have identified a nonsense variant in the gene Muscular LMNA-Interacting Protein (*MLIP*) in a patient affected with adult-onset distal myopathy. MLIP is highly expressed in muscle, but its role hasn't been completely elucidated yet. However, it is known to be the direct interactant of *LMNA* (Lamin Type A/C), which has been linked with laminopathies, cardiomyopathies and muscular dystrophies.

Material and methods: The combination of DNA- and RNA-sequencing led to the identification of the defective *MLIP* gene. Isoform specific analysis are being performed using targeted long-read sequencing.

Results: A homozygous nonsense variant in exon 5 of *MLIP*, an alternatively spliced exon, has been identified. Differential expression showed downregulation of specific *MLIP* transcripts, those containing the *LMNA* interacting site and the NLS sequence; suggestive of a mislocalization and a loss of function. As a compensatory mechanism, we observed an upregulation of its interactant *LMNA*.

Conclusion: Our results highlight the importance of considering alternatively spliced isoforms when calling variants and interpreting their potential functional impact. The interpretation of pathogenicity when considering alternatively spliced isoform will have a direct impact on the affected individuals and their families, it will also help inform on the normal function of *MLIP* in skeletal muscles.

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P10.017.A systematic analysis of genetic variation of duchenne muscular dystrophy and implication for cancer

Hubert Chen

Ivymind Academy, West Windsor, NJ, USA.

Introduction: Duchenne muscular dystrophy (DMD) is a rare, severe, progressive genetic disorder causing disability and premature death. In this study, we performed a systematic analysis of the DMD genetic variants via dbSNP database and explored protein-protein interactions (PPI) for genetic modifiers identified in DMD patients. In addition, DMD genetic alternations in different tumors have also been investigated.

Methods: The genetic variants of DMD genes were extracted from the dbSNP database. PPI map for genetic modifiers identified in DMD patients was constructed using STRING v11. Genetic alternations in the DMD gene with cancer was examined by using cBioPortal.

Results: We examined a total of 3,627 exonic SNPs in the DMD gene. SNPs distributed across all exons. The largest category was nonsynonymous account for nearly 64% of all mutations. Exon 19 appeared to have most density of pathogenic SNP distribution. Nonsense mutation (i.e. stopgain) or frameshift mutation likely lead to more pathogenic. Among the genetic modifiers identified in DMD patients, THBS1 has higher network topological parameters. In addition, we also observed significant poorer overall survival for cancer patients with DMD mutations.

Conclusions: We conducted a systematic genetic analysis of all variants, especially SNPs, in one of the *largest* known human *gene*. Network analysis highlighted non-random interconnectivity between the genetic modifiers identified in DMD patients, and potentially shed light on new genetic modifiers by their functional coupling to these known genes. Our results also suggest DMD gene may serve as a diagnostic and therapeutic target for certain types of cancer.

H. Chen: None.

P10.018.B Newborn screening of Duchenne Muscular Dystrophy specifically targeting deletions amenable to exon-skipping therapy

Pablo Beckers[1], *Jean-Hubert Caberg*[1], *Vinciane Dideberg*[1], *Johan T. den Dunnen*[2], *Tamara Dangouloff*[3], *Vincent Bours*[1], *Laurent Servais*[4], ***François Boemer***[1]

[1]*CHU Liege, Liege, Belgium,* [2]*Leiden University Medical Center, Leiden, Netherlands,* [3]*University of Liege, Liege, Belgium,* [4]*University of Oxford, Oxford, United Kingdom.*

Duchenne Muscular Dystrophy (DMD) is a lethal progressive muscle-wasting disease. New treatment strategies relying on *DMD* gene exon-skipping therapy have recently been approved and about 30% of patients could be amenable to exon 51, 53 or 45 skipping. We evaluated the spectrum of deletions reported in DMD registries, and designed a method to screen newborns and identify *DMD* deletions amenable to exon 51, 53 and 45 skipping. We developed a multiplex qPCR assay identifying hemi(homo)-zygotic deletions of the flanking exons of these therapeutic targets in *DMD* exons (ie. exons 44, 46, 50, 52 and 54). We conducted an evaluation of our new method in 51 male patients with a DMD phenotype, 50 female carriers of a *DMD* deletion and 19 controls. Studies were performed on dried blood spots with patient's consent. We analyzed qPCR amplification curves of controls, carriers, and DMD patients to discern the presence or the absence of the target exons. Analysis of the exons flanking the exon-skipping targets permitted the identification of patients that could benefit from exon-skipping. All samples were correctly genotyped, with either presence or absence of amplification of the target exon. This proof-of-concept study demonstrates that this new assay is a highly sensitive method to identify DMD patients carrying deletions that are rescuable by exon-skipping treatment. The method is easily scalable to population-based screening. This targeted screening approach could address the new management paradigm in DMD, and could help to optimize the beneficial therapeutic effect of DMD therapies by permitting pre-symptomatic care.

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P10.019.C Whole genome sequencing and RNA analysis allow genetic diagnosis of DMD atypical mutations

Rita Selvatici[1], *Rachele Rossi*[1], *Fang Mingyan*[2], *Jerry Lu*[2], *Valeria Sansone*[3], *Maria Sofia Falzarano*[1], *Francesca Gualandi*[1], *Luca Bello*[4], *Elena Pegoraro*[4], *Alessandra Ferlini*[1]

[1]*Dept. of Medical Sciences, Unit of Medical Genetics, University-Hospital of Ferrara, Ferrara, Italy,* [2]*BGI, Shenzen, China,* [3]*Center for the Study of Neuromuscular Diseases, Milan, Italy,* [4]*Dept. of Neurosciences, Unit of Neurology, Padua, Italy.*

Introduction: Dystrophin (DMD) gene mutations cause Duchenne and Becker muscular dystrophies and are routinely identified by MLPA and sequencing. Nevertheless, about 1% of patients remained undiagnosed. Whole Genome Sequencing (WGS) is a high throughput method able to detect theoretically all variant types, including copy number variations or complex genomic rearrangements. FluiDMD is a custom TaqMan based assay we designed to profile the full DMD transcript.

Materials and Methods: We performed FluiDMD and WGS in two DMD cases in which no dystrophin mutations were found. RNA was obtained from urinary stem cells from the two patients.

Results: In the first patient, FluiDMD RNA analysis suggested the occurrence of a genomic event affecting the splicing of exons 54 and 55. WGS identified a large inversion of 15Mb encompassing the region Xp:g.[16147177_31662545inv] with breakpoints in DMD intron 54 and in GRPR (a DMD downstream gene, OMIM*305670) intron 1. In the second patient, FluiDMD analysis showed lack of DMD transcript spanning exon 1-53, with the sole Dp71 isoform expressed. WGS analysis revealed a large inversion of 10Mb encompassing the region Xp:g.[23607043_32981797inv] with breakpoints in DMD intron 2 and in an upstream intergenic, non-coding, region localized in Xp22.12. Both inversions generated fusion genes and DMD-GRPR inversion also generated two fusion transcripts.

Conclusions: FluiDMD RNA profile accurately addresses the gene region where atypical mutations may have occurred and WGS accurately identified the two inversions. We suggest that this diagnostic pipeline might facilitate the identification of undetected genetic variations.

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P10.021.A Lack of correlation between DMDexon 2 duplication splicing choices and phenotype severity

Martina Mietto[1], *Marina Fabris*[1], *Rosa Bonaccorso*[2], *Stefano Carlo Previtali*[2], *Alberto Zambon*[2], *Francesca Gualandi*[1], *Rachele Rossi*[1,3], *Maria Sofia Falzarano*[1], *Alessandra Ferlini*[1,3]

[1]*Unit of Medical Genetics, Department of Medical Sciences, University of Ferrara, Ferrara, Italy,* [2]*Neuromuscular Repair Unit, Head InSpe and Division of Neuroscience, IRCCS Ospedale San Raffaele, Milano, Italy,* [3]*The Dubowitz Neuromuscular Centre, UCL Great Ormond Street Institute of Child Health, London, United Kingdom.*

Introduction: Duchenne muscular dystrophy (DMD) is an X-linked disorder caused by mutations in the *DMD* gene. About 75% of *DMD* mutations are deletions or duplications while the remaining are small mutations. Exon 2 duplication is the most frequent duplication and is associated with variable phenotypes, ranging from mild to severe.

Materials and Methods: We profiled the muscle biopsy splicing pattern of *DMD* exon 2 duplication in six DMD patients (identified by MLPA) using the Agilent High Sensitivity assay. Two patients showed classical DMD phenotype, with loss of ambulation within 13-year-old while in the other four patients ambulation was lost >age 15.

Results: We found four different transcripts due to different splicing choices: the exon2-exon2 duplicated transcript (79%), the intron1-exon2-exon2 transcript, which incorporates an upstream region of intron 1, generating a premature stop codon (14%), a transcript with both exon 2 skipped (3%) and a transcript skipping one exon 2, which generates a normal messenger (6%), this last represented in all six DMD muscles.

Conclusions: We did not identify a phenotype-specific DMD splicing pattern in the six exon 2 duplicated DMD patients. All DMD patients show a similar percentage of wild type transcript (6%), and the very low amount of the transcript skipping both exon 2 suggests that dystrophin rescue due to the alternative ATG translation start site in exon 5 may not play a major role in disease severity. Our results therefore indicate lack of correlation between DMD exon 2 duplication splicing choices and DMD phenotype.

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P10.022.B Somatic mosaicism for Duchenne muscular dystrophy in an asymptomatic 4 year-old boy

Ana-Maria Meašić[1], *Ivona Sansović*[1], *Adriana Bobinec*[1], *Leona Morožin Pohovski*[2], *Mijana Kero*[1], ***Ljubica Odak***[1], *Ingeborg Barišić*[1]

[1]*Department of Medical Genetics and Reproductive Health, Children's Hospital Zagreb, Scientific Centre of Excellence for Reproductive and Regenerative Medicine (CERRM), University of Zagreb School of Medicine, ZAGREB, Croatia,* [2]*Department of Medical Genetics and Reproductive Health, Children's Hospital Zagreb, University of Zagreb School of Medicine, ZAGREB, Croatia.*

Introduction: Duchenne/Becker muscular dystrophies (DMD/BMD) are X-linked recessive disorders caused by mutations in *DMD* gene, characterized by progressive symmetric skeletal muscles weakness, elevated creatine kinase (CK) concentrations and late-onset dilated cardiomyopathy. Here we report a case of a boy with elevated CK and mosaic *DMD* mutation.

Methods: An asymptomatic 4-year-old boy without family history of neuromuscular disorders was referred to us due to incidental findings of elevated CK (2160-3335 U/L). MLPA for *DMD* was performed using peripheral blood DNA, followed by NGS of the clinical exome.

Results: MLPA revealed a change in exon 10 of *DMD*. NGS identified mosaic nonsense mutation c.[986=/C>A] in exon 10 of *DMD* with 70% of reads containing alternative allele A.

Conclusions: The *DMD* shows a high germ-line mutation rate, which predicts high somatic mutation rate and somatic mosaicism. Only eight cases of somatic *DMD* mosaicism are published to date. Somatic mutation rates may be lower than expected, or the patients aren't diagnosed due to mild/uncharacteristic symptoms. Published cases show lower mutation ratio in muscles than in blood, consistent with a genetic normalization, a selective pressure against mutant muscle cells. This could explain the lack of symptoms in our case. Patient's young age should also be considered as he could develop symptoms later in life. This study

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P10.023.C Dystrophin isoforms transcription heatmap in human adult control brain areas

Maria Sofia Falzarano[1], *Rachele Rossi*[1,2], *Martina Mietto*[1], *Fernanda Fortunato*[1], *Rita Selvatici*[1], *Pietro Spitali*[3], *Federica Montanaro*[2], *Francesco Muntoni*[2], *Alessandra Ferlini*[1,2]

[1]*Unit of Medical Genetics, Department of Medical Sciences, University of Ferrara, Ferrara, Italy,* [2]*The Dubowitz Neuromuscular Centre, UCL Great Ormond Street Institute of Child Health, London, United Kingdom,* [3]*Department of Human Genetics, Leiden University Medical Center, Leiden, Netherlands.*

Introduction: Duchenne muscular dystrophy (DMD) is an X-linked disease due to pathogenic variants in the *DMD* gene. In addition to the neuromuscular involvement, DMD often presents with cognitive and neuro-behavioural co-morbidities, for which the pathogenesis and genotype-phenotype relationship are partially understood. Multiple *DMD* isoforms, differentially affected based on the site of the mutation, are thought to play a relevant role in these co-morbidities, based on their prominent (Dp71) or exclusive (Dp140) brain expression.

Material and methods: All known dystrophin isoforms were analysed in 24 normal adult human brain areas using TaqMan assays on TissueScan cDNA array (OriGene). Ct were used to build a heatmap of dystrophin isoforms expression with values ranging from 40 (lowest) to 27 (highest) Ct.

Results: Three main dystrophin isoform clusters were identified. Cluster 1 includes Dp116, Dp427p1 and Dp260.1 that show low expression in all brain areas. Cluster 2 includes Dp260.2, Dp427m, Dp140 with intermediate expression levels. Cluster 3 consists of Dp427b, Dp427p2, Dp71 that have the overall high expression in most brain areas. Hierarchical clustering also highlighted brain areas with overall low dystrophin expression, such as telencephalon (frontal, temporal and occipital lobe, amygdala, caudate, and choroid plexus), diencephalon (thalamus and hypothalamus), and myelencephalon (medulla). By contrast, the cerebellum showed the highest expression of Dp140.

Conclusion: The approach showed that adult human brain areas show differential enrichment for expression of specific dystrophin isoforms. This information may help understand the role of dystrophin in brain co-morbidities observed in affected DMD patients. Grant: BIND EU Grant N. 847826

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P10.024.D Whole genome sequencing - A solved case of dystrophinopathy in a young girl

Natalie Pluta[1], *Ann-Kathrin Zaum*[1], *Arpad von Moers*[2], *Indrajit Nanda*[1], *Frederic Zimmer*[1], *Cosima Drewes*[1], *David Atlan*[3], *Beat Wolf*[4], *Simone Rost*[1]

[1]*Institut fuer Humangenetik, Wuerzburg, Germany,* [2]*DRK Kliniken, Berlin, Germany,* [3]*Phenosystems SA, Wallonia, Belgium,* [4]*iCoSys, Fribourg, Switzerland.*

Introduction: Whole Genome Sequencing (WGS) will be the method of choice in near future genetic diagnostics. Major challenges in WGS are interpreting and handling the large amount of data and finding the causative variant(s). Here we present a case that was solved with WGS in which all other methods used did not yield definite results.

Material/Methods: The patient is a 6 year old girl with muscle weakness and very high levels of serum creatine kinase (CK) >20,000U/l. Due to her symptoms and an almost completely skewed X-inactivation a dystrophinopathy was strongly suggested. WGS was performed and analyzed with our software for routine NGS diagnostics (GensearchNGS, Phenosystems).

Results: We found a position in intron 54 of the *DMD* gene where a small intergenic sequence of chromosome (chr) 5 was detected. By performing Sanger sequencing we confirmed a reciprocal translocation involving chr 5p and Xp with definite breakpoints. Previously performed cytogenetic analysis could not depict the evidence of a translocation between 5p and Xp. An extended FISH analysis could validate the translocation and will be useful to demonstrate the participation of the active chr X in the translocation.

Discussion: This case shows how important WGS will be or already is for genetic routine diagnostics. By performing WGS as a first step this case could have been solved faster and with higher accuracy than the stepwise application of conventional methods.

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P10.025.A Bionano optical genome mapping and southern blot analysis for FSHD detection

Jeroen Depreeuw, Barbara Dewaele, Sascha Vermeer, Gert Matthijs, ***Valérie Race***

Center for Human Genetics, University Hospital of Leuven, Leuven, Belgium.

Introduction: Facioscapulohumeral muscular dystrophy (FSHD; OMIM*158900) is an autosomal dominant muscular disorder characterized by slowly progressive dysfunction of facial, upper and lower extremity muscles. FSHD1 is associated with the contraction of the D4Z4 microsatellite in the 4q35 subtelomeric region. The most common technique for genetic diagnosis of FSHD1 is Southern Blotting. We explored the potential of a new technique, called Optical Genome Mapping (OGM) from Bionano Genomics for FSHD1 detection.

Materials and Methods: Blood samples from 10 patients with FSHD related symptoms were collected and stored at -80°C. OGM was performed according to the manufacturer's instructions and samples were loaded on a Saphyr instrument. Bionano "EnFocus FSHD" pipeline was used to calculate the haplotype and the number D4Z4 repeats (diagnostic when shorter than 10 repeats). OGM results were then compared with the results from the Southern Blot analyses.

Results: Patient samples included 2 affected individuals (7 and 8 repeats) and 8 unaffected individuals (repeat range 13-59). OGM results were concordant with Southern Blot results. There were no false positives nor false negatives in our series and no differences were found in terms of repeatability and reproducibility. In addition, OGM could differentiate between 4qA (pathogenic) and 4qB (not pathogenic) haplotypes.

Conclusions: We conclude that OGM is a powerful and robust technique for FSHD1 testing in genetic diagnostic laboratories, providing results that are completely concordant with the current gold standard Southern Blot analysis. Additionally, OGM provides extra relevant information, such as the 4qA or 4qB haplotype and *SMCHD1* structural variations.

J. Depreeuw: None. **B. Dewaele:** None. **S. Vermeer:** None. **G. Matthijs:** None. **V. Race:** None.

P10.026.B A family with mutation in *DMD* that inherited from gonadal mosaic mother

Basak Gogus, *Muhsin Elmas*

Afyonkarahisar Health Science University, Afyonkarahisar, Turkey.

Introduction: Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are X-linked recessive disorders these affect 1 in 7,250 males aged 5 - 24 years. Mutations in the *Dystrophin* (*DMD*) cause DMD, BMD. Mostly, female relatives of patient's are carrier. Clinical symptoms are delayed motor milestones like walking independently. In addition, increasing at creatinine kinase occur at these patients. In genetics, one of most challenging handicap is gonadal (germline) mosaicism mutations. Diseases caused by germline mosaicism can be difficult to diagnose as genetically-inherited because the mutant alleles are not likely to be present in the somatic cells. We aim to present one family with germline mosaicism in DMD gene.

Materials and Methods: 18 months old girl was referred our clinic because of increasing creatinine kinase that found incidentally. She had one sister. We decide to perform DMD MLPA test for her. At the same time, if we will find any pathological changes, same test will be performed for her sister and mother.

Results: At the result of DMD MLPA analysis of patient, we determined heterozygous deletion at 14th and 15th exons. The result of her sister was same with proband. But their mother didn't had any changes in DMD gene. This situation gave us the idea of a possible presence of gonadal mosaicism in the mother.

Conclusions: If there is repating for same mutation but parents haven't changes, gonadal mosaicm consider. While giving genetic counseling, parents should be informed about the rate at which their next child becomes patient.

B. Gogus: None. **M. Elmas:** None.

P10.027.C A novel *de novo* missense *NIPA1* mutation causing hereditary spastic paraplegia

Dora Fabbro[1], *Catia Mio*[2], *Federico Fogolari*[2], *Giuseppe Damante*[2]

[1]*Azienda Sanitaria Universitaria Friuli Centrale, Udine, Italy,* [2]*University of Udine, Udine, Italy.*

Introduction: Hereditary Spastic Paraplegia (HSP) includes a heterogeneous group of neurodegenerative disorders characterized by spasticity, hyperreflexia and weakness in the lower limbs, due to the degeneration of corticospinal axons. To date, more than 80 types of HSP have been identified. SPG6 accounts for 1% of autosomal dominant HSP and is caused by pathogenic variants in *NIPA1* (OMIM# 608145). It encodes a magnesium transporter located in plasma membrane and early endosomes, implicated in neuronal development and maintenance. Here we report a 39-years-old woman affected by progressive gait disturbance associated to absence seizures episodes within childhood.

Materials and Methods: Clinical exome sequencing was performed identifying a *de novo* heterozygous variant in *NIPA1* (NM_144599.5 c.249C>G; p.Asn83Lys). Molecular modelling was performed to evaluate putative functional consequence of the NIPA1 protein.

Results: The *NIPA1* c.249C>G mutation is classified as likely pathogenic following the ACMG (American College of Medical Genetics and Genomics)/AMP (Association for Molecular Pathology) guidelines. The Asn83 is located between the second transmembrane domain (TMD) and a loop exposed to the extracellular space. By molecular modelling, the Asn83Lys modification induces a significant perturbation of the protein structure, altering signal transduction or small-molecule transport by modulating the length of TMDs.

Conclusion: We described a novel Asn83Lys mutation in NIPA1 in a patient displaying the chief phenotypical characteristics of SPG6. Our data are in agreement with the idea that *NIPA1* missense variants in SPG6 act through a gain of function mechanism able to activate the apoptotic program, as suggested by studies performed in C. Elegans.

D. Fabbro: None. **C. Mio:** None. **F. Fogolari:** None. **G. Damante:** None.

P10.030.B Clinical and genetic characterization of seven Portuguese patients with *KMT2B* variants

Alexandra M. Lopes[1], *Susana Sousa*[1], *Ana Lopes*[1], *Paulo Silva*[1], *Sara Morais*[1], *Ana F. Brandão*[1], *Fátima Lopes*[1], *Rita Bastos-Ferreira*[1], *André Jorge*[2], *Cristina Januário*[2], *Henrique M. Costa*[3], *Ana C. Brás*[2], *Rita Quental*[4], *Miguel Leão*[4], *Joana Damásio*[1,5], *Marina Magalhães*[5], *João P. Freixo*[1], *Jorge Sequeiros*[1], *Jorge Oliveira*[1]

[1]*CGPP-IBMC/I3S, Porto, Portugal,* [2]*Serviço de Neurologia, Centro Hospitalar e Universitário de Coimbra, EPE, Coimbra, Portugal,* [3]*Serviço de Neurologia, Centro Hospitalar de Vila Nova de Gaia/Espinho, EPE, Vila Nova de Gaia, Portugal,* [4]*Serviço de Genética Médica, Centro Hospitalar Universitário de São João/Faculdade de Medicina da Universidade do Porto, Porto, Portugal,* [5]*Centro Hospitalar Universitário do Porto, Hospital de Santo António, Porto, Portugal.*

KMT2B pathogenic variants were recently identified as an important cause of early-onset dystonia. The phenotype spectrum of *KMT2B*-related diseases is expanding as more complex neurological and syndromic manifestations emerge. Clinical and genetic data from seven Portuguese patients with causal variants in *KMT2B* were reviewed to further characterize its mutational spectrum and reassess phenotype. Two patients presented isolated dystonia, while five had more complex or atypical phenotypes. NGS multigene panels, based on whole-exome sequencing, were applied in six patients, whereas one was directly tested for variants in *KMT2B* by Sanger sequencing, according to the clinical request. The two patients with isolated dystonia carried one missense variant of unknown clinical significance (p.Arg1762His) or one splice-site variant (c.5198-4_5206del). The latter occurred *de novo*; neither had been previously reported. In the group of patients with complex dystonia, one frameshift (p.Glu1267Alafs*35) and two missense variants (p.Arg145Gln and p.Arg1777Pro) were identified. Two were pathogenic or likely-pathogenic, whereas one missense substitution was of unknown clinical significance. Only one had been reported. One patient with an atypical phenotype, comprising ataxia, subtle dystonia and polyneuropathy, harboured a novel splice-site variant (c.3334+1G>A). One additional patient with a syndromic presentation, including dysmorphic features and intellectual disability, but no dystonia, carried a novel frameshift variant (p.Ala1856Profs*115); its clinical significance and origin are under investigation as another

candidate variant in *TRIO* was found in this patient. We expand the spectrum of pathogenic variants in *KMT2B* and hope contributing to explore further their association with atypical and syndromic phenotypes.

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P10.031.C Partial uniparental disomy of chromosome 4 causes by homozygous TRAPPC11 truncating variant

Nawale HADOUIRI[1,2], *Véronique Darmency*[3], *Véronique Dulieu*[2], *Marie-Gabrielle Mourot De Rougemont*[4], *Benoit Collomb*[5], *Stéphanie Perez Martin*[6], *Olivier Blanchard*[7], *Antonio Vitobello*[8], *Christophe Philippe*[9], *Laurence Faivre*[1,10], *Christel Thauvin-Robinet*[1,11,12]

[1]*UMR1231 GAD, Inserm - Université Bourgogne-Franche Comté, Dijon, France,* [2]*Pôle rééducation-réadaptation, CHU de Dijon, 23, rue Gaffarel, Dijon, France,* [3]*3- Service de Neurophysiologie Clinique, Hôpital d'Enfants, CHU Dijon Bourgogne, Dijon, France,* [4]*4- Hôpital Francois Mitterrand, département de radiologie diagnostique et thérapeutique, CHU, 14, rue Paul Gaffarel, Dijon, France,* [5]*5- Unité de Réanimation polyvalente, Hôpital d'Enfants, CHU Dijon Bourgogne, Dijon, France,* [6]*Pôle pédiatrie, Hôpital d'Enfants, CHU Dijon Bourgogne, Dijon, France,* [7]*Service de Pédiatrie, Centre Hospitalier de Roanne, Roanne, France,* [8]*Unité Fonctionnelle Innovation en Diagnostic génomique des maladies rares, FHU-TRANSLAD, Dijon, France,* [9]*8- Unité Fonctionnelle Innovation en Diagnostic génomique des maladies rares, FHU-TRANSLAD, Dijon, France,* [10]*9- Centre de Référence maladies rares « Anomalies du Développement et syndromes malformatifs », centre de génétique, FHU-TRANSLAD, CHU Dijon Bourgogne, Dijon, France,* [11]*10- Centre de Référence maladies rares « Déficiences Intellectuelles de causes rares », CHU Dijon Bourgogne, Dijon, France,* [12]*Unité Fonctionnelle Innovation en Diagnostic génomique des maladies rares, FHU-TRANSLAD, CHU Dijon Bourgogne, Dijon, France.*

Introduction: Transport protein particle (TRAPP) is a multisubunit tethering complex implicated in tricellular vesicle trafficking. Biallelic pathogenic variants of the *TRAPC11* gene have rarely been associated with various phenotypes from limb-girdle muscular dystrophy (LGMD) to congenital disorder of glycosylation (CDG), associated to various extramuscular symptoms. To date, only 20 patients have been reported in the literature with 13 pathogenic variants.

Patients and Results: In a 15-month-old male referred for severe developmental delay, weakness of the 4 limbs and pelvic girdle muscles, mild dysmetria of the upper limbs, severe microcephaly, seizures, and mildly elevated creatine kinase (670 UI/L), trio exome sequencing (ES) identified a homozygous pathogenic splice-site variant (NM_021942.5:c.1287+5G>A) in the intron 12 of the *TRAPPC11* gene, only inherited from his father. Targeted ES reanalysis identified a paternal partial 24 Mb uniparental disomy (UPD) of the chromosome 4 including this variant, previously reported in 5 affected members. Affected individuals had microcephaly, early-onset psychomotor delay, hyperkinetic movement disorder, truncal ataxia, and elevated creatine kinase (300−1200 UI/L). At last follow-up (4 years of age), he presented a persistent muscular weakness, severe microcephaly, seizures, delayed developmental milestones and thin corpus callosum. His muscular phenotype appeared more pronounced that previous reported in cases with the same *TRAPPC11* variant.

Conclusion: This report highlights the benefit of trio ES for the precocious diagnosis of children affected with severe developmental delay and unusual causative molecular mechanisms such as partial UPD. It also appears highly useful for genetic counseling for parents to precise the recurrence risk.

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P10.033.A Homozygous splice-site variant in *MADD,* encoding a Rab guanine nucleotide exchange factor, results in pleiotropic effects and a multisystemic infantile-lethal disorder

Hagar Mor-Shaked[1], *Bassam Abu-Libdeh*[2], *Amir A. Atawna*[3], *Gillis David*[4], *Orli Halstuk*[1], *Nava Shaul-Lotan*[1], *Mordechai Slae*[4], *Mutaz Sultan*[5], *Vardiella Meiner*[1], *Orly Elpeleg*[1], *Tamar Harel*[1]

[1]*Department of Genetics, Hadassah-Hebrew University Medical Center, jerusalem, Israel,* [2]*Department of Pediatrics, Makassed Hospital and Faculty of Medicine, Al-Quds University, East Jerusalem, Israel,* [3]*Department of Neonatology, Makassed Hospital, East jerusalem, Israel,* [4]*Department of Pediatrics, Hadassah-Hebrew University Medical Center, jerusalem, Israel,* [5]*Department of Pediatrics, Makassed Hospital and Faculty of Medicine, Al-Quds University, East jerusalem, Israel.*

Introduction: Rab proteins coordinate inter-organellar vesicle-mediated transport, facilitating intracellular communication, protein recycling, and signaling processes. Dysfunction of Rab proteins or their direct interactors lead to a wide range of diseases with diverse manifestations. *MADD* encodes a Rab guanine nucleotide exchange factor (GEF) which activates RAB3 and RAB27A/27B and is thus a crucial regulator of neuromuscular junctions and endocrine secretory granule release. Pathogenic variants in *MADD* have recently been associated with syndromic neurodevelopmental disorder [MIM 619005] and with DEEAH syndrome [MIM 619004].

Materials and Methods: Following informed consent, exome sequencing analysis was undertaken on seven individuals from four consanguineous Arab Muslim families with an infantile-lethal syndrome including failure to thrive, chronic diarrhea, neonatal respiratory distress, variable pituitary dysfunction and distal arthrogryposis. Sanger sequencing was used to segregate the candidate variant in available family members, and analysis of cDNA allowed characterization of the mutant transcript. Result: Internal gene-matching using a local exome database allowed the identification of a homozygous splice-site variant in *MADD* (c.2816 +1G>A) on a common haplotype in all four families, indicating a founder variant. The variant segregated with the disease in all available family members. Determination of cDNA sequence verified single exon skipping, resulting in an out-of-frame deletion.

Conclusions: The combined roles of *MADD* and its downstream effectors correlate with the phenotypic spectrum of disease, and call for additional studies to confirm the pathogenic mechanism and to investigate possible therapeutic avenues through modulation of TNF-α signaling. The authors declare no funding sources for this project.

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P10.034.B Variants in the imprinted gene *MAGEL2*

Evelyn Douglas[1], *Louisa Sanchez*[1], *Jessica Burdett*[2], *Shannon Le Blanc*[3], *Nicholas J. C. Smith*[4], *Andrew Dubowsky*[2], *David Lawrence*[5], *Karin Kassahn*[6], *Sui Yu*[1], *Kathie Friend*[1]

[1]SA Pathology (Women's and Children's Hospital), North Adelaide, Australia, [2]SA Pathology (Flinders Medical Centre), Bedford Park, Australia, [3]Paediatric and Reproductive Genetics Unit (Women's and Children's Hospital), North Adelaide, Australia, [4]Neurology (Women's and Children's Hospital), North Adelaide, Australia, [5]CCB ACRF Cancer Genomics Facility, Adelaide, Australia, [6]SA Pathology (Technology Advancement Unit), Adelaide, Australia.

Pathogenic variants in *MAGEL2* are associated with Chitayat-Hall (CHS), Schaaf-Yang (SYS) and Prader-Willi (PWS) syndromes. Schaaf-Yang Syndrome is characterised by neonatal hypotonia, developmental delay, intellectual disability, feeding problems in infancy, joint contractures and autism spectrum disorder, sharing clinical overlap with Prader-Willi Syndrome and Chitayat-Hall syndrome. Truncating variants in the maternally imprinted *MAGEL2* gene have been identified in patients with SYS.

A neuromuscular gene panel analysis was requested on a two week old male infant, requiring respiratory support, with hypotonia, bilateral talipes and central apnoeas. No variants explaining the patient's phenotype were initially found, but subsequent reanalysis of the exome identified a truncating variant in the *MAGEL2* gene. Familial studies showed this variant to be paternally inherited and also confirmed this variant in the paternal grandmother, consistent with the inheritance pattern of Schaaf-Yang Syndrome due to imprinted inheritance.

Following this finding, the *MAGEL2* gene was added to our standard neuromuscular panel. To date, only one additional variant has been identified in *MAGEL2*, an in-frame deletion of 30 nucleotides. Parental studies indicated this variant to be maternally inherited, so it is considered unlikely to be disease causing.

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P10.035.C Phenotypic variability of *MEGF10* variants causing congenital myopathy Report of two unrelated patients from a highly consanguineous population

Hanan Suliman AlQudairy, *Mohammad AlMuhaizea, Omar Dabbagh, Wafa Alotaibi, Aljouhra AlHargan, Mariam Ali, Hindi AlHindi, Dilek Colak, Namik Kaya*

King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia.

Congenital mypoathies are rare neuromuscular hereditary disorders that manifest at birth or during infancy and usually appear with muscle weakness and hypotonia. Early onset myopathy, areflexia, respiratory distress, and dysphagia (EMARDD, OMIM: 614399, MIM: 612453) is a rare autosomal recessive disorder caused by biallelic mutations (at homozygous or compound heterozygous status) in *MEGF10* (multiple epidermal growth factor-like domains protein family). Here, we report two unrelated patients, born to consanguineous parents, having two novel *MEGF10* mutations. There are only five cases reported in the literature to date. Interestingly, presence of *MEGF10* associated EMARDD has not been reported in the Saudi Arabia, a highly consanguineous population. Our work expands phenotypic features of the disease and provides opportunity for genetic counseling to the inflicted families. Awareness of the implications of consanguineous union is extremely important for parental counseling and recognition in a greatly inbred society.

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P10.036.D Mitochondria-lysosome membrane contacts are defective in *GDAP1*-related Charcot-Marie-Tooth disease

Lara Cantarero[1,2], *Elena Juárez-Escoto*[1], *Azahara Civera-Tregón*[1,2], *María Rodríguez-Sanz*[1], *Yaiza Díaz-Osorio*[1], *Mónica Roldán*[1,3], *Raúl Benítez*[1,4], *Janet Hoenicka*[1,2], *Francesc Palau*[1,2,3]

[1]Laboratory of Neurogenetics and Molecular Medicine – IPER, Institut de Recerca Sant Joan de Déu, Barcelona, Spain, [2]CIBER de Enfermedades Raras (CIBERER), Barcelona, Spain, [3]Dept. of Genetic Medicine – IPER, Hospital Sant Joan de Déu, and Hospital Clínic, Universitat de Barcelona, Barcelona, Spain, [4]Automatic Control Department and Biomedical Engineering Research Center, Universitat Politècnica de Catalunya, Barcelona, Spain.

Introduction: Mitochondrial membrane contact sites (MCSs) allow bidirectional communication between mitochondria and other organelles for specific functions. GDAP1 is an atypical glutathione S-transferase located both in the outer mitochondrial membrane and in the mitochondrial contacts with endoplasmic reticulum (MAMs). Since mutations in GDAP1 cause Charcot-Marie-Tooth neuropathy, its function is essential for peripheral nerve physiology. Our previous studies showed structural defects in mitochondria and ER cisternae, associated with abnormal autophagic vesicles. Nevertheless, the underlying pathophysiological mechanisms of loss of function mutations remain elusive.

Materials and methods: Cellular and functional experiments on autophagy, interorganelles MCSs and mitochondrial network in embryonic motor neurons from *Gdap1*$^{-/-}$ mice, *GDAP1*-silenced SH-SY5Y cells and *GDAP1* patients' fibroblasts.

Results: We demonstrate that GDAP1 participates in basal autophagy and its depletion affects autophagosome biogenesis and membrane trafficking from MAMs. GDAP1 also participates in lysosome maturation by interacting with PYK*fyve*, a pH-dependent kinase. Notably, we present GDAP1-LAMP-1 as a new tethering pair of mitochondria-lysosome MCSs. GDAP1 deficiency reduces MCSs between these organelles, impairs lysosomal and mitochondrial network morphology and decreases cellular glutathione levels. Supplying this antioxidant rescues lysosomes membrane and mitochondrial dynamics defects but not early autophagic events or mitochondrial MCSs.

Conclusions: GDAP1 enables the proper function of mitochondrial MCSs in both degradative and non-degradative pathways, which could explain primary insults in *GDAP1*-related CMT pathophysiology and highlights new redox-sensitive targets in axonopathies where mitochondria and lysosomes are involved. **Grants:** Spanish Ministry of Science, Innovation and Universities (SAF2015-66625-R), Generalitat de Catalunya 2015 FEDER/S-21, and CIBERER.

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P10.037.A Cis MFN2 missense variants causing familial CMT2A2A

Enrica Marchionni[1], *Fabiana Fattori*[2], *Gioia Mastromoro*[1], *Daniele Guadagnolo*[1], *Francesca Di Palma*[1], *Luca Bosco*[2], *Enrico Bertini*[2], *Antonio Pizzuti*[1]

[1]*Department of Experimental Medicine, Sapienza University of Rome, Rome, Italy,* [2]*Unit of Neuromuscular and Neurodegenerative Disorders, Department of Neurosciences, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy.*

Introduction: MFN2 (MIM*608507) is a nuclear gene encoding for a mitochondrial outer membrane protein. Heterozygous mutations in MFN2 are associated with Charcot-Marie-Tooth (CMT) disease type 2A2A (CMT2A2A MIM*609260) and CMT6A (MIM*601152) while biallelic variants cause CMT2A2B (MIM*617087).

Materials and Methods: A 39 y.o. woman was referred for a peripheric neuropathy, sensory deficits and distal-predominant weakness. ENMG showed a marked neurogenic involvement. At birth she presented clubfeet and during childhood she underwent nine surgical interventions for foot and ankle deformities. She also presented scoliosis and a severe symptoms progression. DNA was extracted from peripheral blood and Next Generation Sequencing (NGS) was performed using an Illumina MiSeq analyzing a customized gene panel including 111 genes related to peripheral neuropathies and/or distal SMA. Target enrichment was carried out using Nextera Rapid Capture Custom Enrichment Kit.

Results: NGS analysis detected two heterozygous MFN2 (NM_014874.3) variants: c.2170C>G; p.(Leu724Val) and c.2200C>G; p.(Leu734Val). The first one is absent in GnomAD v2.1.1, clinical databases and is not reported in literature. The second one is described in literature in a patient presenting CMT2A. Segregation analysis disclosed the same variants in her 69 y.o. father, revealing the cis phase. Neurological examination evidenced milder symptoms and the presence of pes cavus, scoliosis and distal weakness.

Conclusions: MFN2 genotype-phenotype studies show significant phenotypic and allelic heterogeneity and variant interpretation is challenging. Interrogation of the in house database permitted to find another family with similar indication analysis and the same in cis variants, currently under clinical reassessment to better refine the associated phenotype.

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P10.038.B Exploring the role of genetic modifiers in a mild LAMA2-RD case associated with a *LAMA2* loss-of-function mutation

Veronica Pini[1], *Ben Weisburd*[2], *Vijay Ganesh*[2], *Stephanie Di Troia*[2], *Francesco Catapano*[1], *Sara Aguti*[1], *Francesco Muntoni*[1]

[1]*UCL Great Ormond Street Institute of Child Health - Dubowitz Neuromuscular Centre, London, United Kingdom,* [2]*Centre for Mendelian Genomics - Broad Institute of MIT and Harvard, Cambridge, MA, USA.*

Introduction: Merosin-deficient congenital muscular dystrophy (LAMA2-RD) is a neuromuscular disorder caused by mutations in the *LAMA2* gene, coding for the alpha-2 subunit of laminin-211 (merosin). LAMA2-RD patients carrying *LAMA2* loss-of-function mutations lack merosin and their invariably severe clinical phenotype is characterised by the inability to acquire ambulation. A wider (often milder) spectrum of disease severity results from missense mutations, if a partially functional protein is produced. To date, only one LAMA2-RD patient was reported with a very mild phenotype despite the total merosin absence in muscle. This patient is still ambulant at the age of 30 with no respiratory insufficiency nor cardiomyopathy. Interestingly, this patient carries the same *LAMA2* loss-of-function mutation as a severely affected sibling. We hypothesized that genetic modifier(s) in the atypical patient mitigate the consequences of complete merosin deficiency via a novel mechanism.

Methods: We performed RNA-sequencing on RNA obtained from muscle samples of the atypical patient, the affected sibling and unrelated LAMA2-RD patients with both total and partial merosin deficiency.

Results: Transcriptome analysis showed a similar *LAMA2* gene expression in the atypical patient, the affected sibling and patients with absent merosin. A limited number of genes differentially expressed in the atypical patient affect pathways potentially relevant for the observed phenotypic divergence.

Conclusion: We plan to study the role of candidate modifier(s)/pathways in dystrophic zebrafish and identify novel mechanisms to attenuate LAMA2-RD severity. New knowledge about the molecular aspects of the disease could lead to the development of new therapeutic approaches for LAMA2-RD. Project supported by CureCMD.

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P10.039.C Interactome-based multi-omics study of molecular networks implicated in disease activity in Multiple Sclerosis

Valentina Nale[1], *Ferdinando Clarelli*[2], *Noemi Di Nanni*[1], *Laura Ferrè*[2,3], *Elisabetta Mascia*[2], *Silvia Santoro*[2], *Matteo Gnocchi*[1], *Marco Moscatelli*[1], *Martina Tosi*[2], *Kaalindi Misra*[2], *Melissa Sorosina*[2], *Luciano Milanesi*[1], *Massimo Filippi*[3,4,5], *Federica Esposito*[2,3], *Ettore Mosca*[1]

[1]*Institute of Biomedical Technologies, National Research Council, Segrate (Milan), Italy,* [2]*Laboratory of Neurological complex disorders, Division of Neurosciences, IRCCS San Raffaele Scientific Institute, Milan, Italy,* [3]*Neurology, Neurorehabilitation and Neurophysiology Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy,* [4]*Vita-Salute San Raffaele University, Milan, Italy,* [5]*Neuroimaging Research Unit, Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Milan, Italy.*

Introduction: Multiple Sclerosis (MS) is an autoimmune disease of the Central Nervous System, characterized by high clinical heterogeneity. Interactome-based approaches to complex diseases have proven successful to fill the gap of knowledge between gene-level findings and pathological phenotypes. In this study, we used tissue-specific as well as general models of the human interactome to find gene networks affected by the most significant molecular alterations associated with disease activity in MS patients.

Materials and Methods: The genome-wide association study involved 1174 patients; gene-wise statistics were estimated with VEGAS; differential expression analysis was performed on whole blood samples from 182 patients. Tissue-specific interactomes source: HumanBase. General interactomes: STRING, DIAMOND, HINT, iRefIndex. Network analysis: "dmfind" (genomics); "mND" (genomics and transcriptomics). Statistical significance was assessed by permutation-based approaches. Pathway sources: Biosystems, MsigDB. Pathway cross-talk was quantified considering inter-pathway gene-gene interactions.

Results: The analysis of genomics in brain-specific and lymphocyte-specific interactomes revealed a series of genes that carry variants and form significant gene networks; these networks share a common component, but let us also hypothesize tissue-specific effects on different pathways. The integrative analysis revealed intra-pathway functional links between the genetic and expression alterations, and pathway cross-talks, with a core of conserved relations (recurrent across interactomes) as well as interactome-specific findings.

Conclusions: Collectively, the gene networks emerging in our study provide an in-depth knowledge of molecular pathways implicated in disease activity. Our study underlines also the importance of integrating complementary approaches to address the complexity of molecular networks. Funding: MoH GR-2016-02363997; FRRB ERAPERMED2018-233 FindingMS GA 779282.

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P10.040.D Expanding disease spectrum of muscular dystrophy

Marwan Nashabat, *Şahin Avcı, Esra Börklü-Yücel, Hilal Pırıl Saraçoğlu, Piraye Oflazer, Nathalie Escande-Beillard, Hülya Kayserili*

Koç University, Istanbul, Turkey.

Congenital muscular dystrophy is a genetically and phenotypically heterogeneous spectrum of disorders characterized by progressive muscle weakness starting at infancy or early childhood. Although currently the majority of patients are diagnosed by high throughput genetic investigations, a large number of muscular dystrophies remain unraveled.

Case presentation: 8-year-old female patient born from a nonconsanguineous marriage developed normally until early childhood. At 5 years old, she started to experience frequent falls and developed an abnormal gait. Her symptoms progressively worsen over time. The patient has normal growth and mental abilities. Her upper and lower limbs muscle power decreased ranging from 2 to 4/5. The patient has waddling gait with mildly anterior pelvic tilt and thoracic scoliosis. Biochemical investigations showed elevated creatinine kinase level. Electromyography showed a myopathic pattern. The muscle biopsy analysis was consistent with muscular dystrophy. All usual genetic investigations including clinical exome sequencing, myositis gene panel, and *DMD* MLPA analysis were unremarkable. Interestingly, whole exome sequencing revealed a promising candidate involved in the nucleo-cytoplasmic trafficking. Segregation analysis results suggest autosomal recessive inheritance. Encouragingly, additional cases with more severe phenotype have been identified by using GeneMatcher. Functional validation is under investigation.

Conclusion: The recent advances in genetic investigations improved substantially the understanding of the pathophysiology of many congenital muscular dystrophies. Further research is needed to unravel new causative candidates and possible treatments for the undiagnosed patients.

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P10.041.A Functional analysis of an RYR1 variant underlying a myopathy with variable expressivity

Jennifer Hauteclocque[1,2], *Adrien Rihoux*[3,2], *Jean-Denis Brisson*[4,5,6], *Alex Parker*[1,2], *Cam-Tu E. Nguyen*[1,7], *Martine Tétreault*[1,2]

[1]*Département de Neurosciences, Université de Montréal, Montreal, QC, Canada,* [2]*Centre de Recherche du CHUM, Montreal, QC, Canada,* [3]*Département de Microbiologie, Infectiologie et Immunologie, Université de Montréal, Montreal, QC, Canada,* [4]*Faculté de médecine et des sciences de la santé, Université de Sherbrooke, Sherbrooke, QC, Canada,* [5]*Groupe de recherche interdisciplinaire sur les maladies neuromusculaires, Centre intégré universitaire de santé et de services sociaux du Saguenay-Lac-St-Jean, Saguenay, QC, Canada,* [6]*Clinique des maladies neuromusculaires, Centre intégré universitaire de santé et de services sociaux du Saguenay-Lac-St-Jean, Saguenay, Saguenay, QC, Canada,* [7]*Pediatric Neurology, CHU Sainte-Justine, Université de Montréal, Montreal, QC, Canada.*

Neuromuscular diseases are known to be highly heterogeneous diseases where a single mutation is often associated with a wide range of phenotypes. Although the nature and location of the variant can explain the variability between different mutations, differences in clinical presentation amongst carriers of the same variant remain elusive. We present a study cohort composed of individuals carrying the same uncharacterized *RYR1* heterozygous missense variant but who demonstrate extremely diverse phenotypes ranging from exertional rhabdomyolysis, fixed weakness with ptosis, and asymptomatic individuals. Our goal is to validate the pathogenicity of this variant before proceeding to investigate the molecular basis of this clinical heterogeneity.

We used a CRISPR/Cas9 mediated homozygous knock-in of the *RYR1* variant in *C. elegans* to study the functional effects of the mutation. Locomotion, lifespan, and synaptic transmission were measured and compared to wild type (wt) controls using video microscopy, a lifespan assay, and an aldicarb-induced paralysis assay respectively. As such, we identified a decrease in mobility, longevity, and synaptic efficacy in mutant worms compared to the wt.

We conclude that this *RYR1* variant is in fact pathogenic as it causes a variety of neuromuscular phenotypes in our *C. elegans* model. This provides further evidence for the pathogenicity of this variant in humans and corroborates the idea that this cohort is ideal to study the mechanisms of clinical heterogeneity. Finally, these findings will not only have a direct impact on the affected individuals and their families, but will also help inform disease progression, risk management and prognosis.

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P10.042.B The c.794C>T p.(Ala265Val) *SCN4A* variant may be associated with congenital myopathy with FSHD-like phenotype

Barbora Plevová[1], *Jana Zídková*[2], *Petra Laššuthová*[3], *Roope Männikkö*[4], *Jana Haberlová*[1]

[1]*Department of Paediatric Neurology, Second Faculty of Medicine (2. LF UK) and University Hospital Motol, Prague, Czech Republic,* [2]*Centre of Molecular Biology and Genetics, University Hospital Brno, Brno, Czech Republic,* [3]*Neurogenetic laboratory, Department of Paediatric Neurology, Second Faculty of Medicine (2. LF UK) and University Hospital Motol, Prague, Czech Republic,* [4]*Department of Neuromuscular Disorders, UCL Queen Square Institute of Neurology, London, United Kingdom.*

Background: The causes and manifestations of congenital myopathy may be diverse. Loss-of-function variants in the *SCN4A* gene are the cause of autosomal recessive (AR) severe foetal hypokinesia and congenital myopathy. Gain-of-function variants were associated with autosomal dominant myotonia and periodic paralysis.

Patients and Methods: A 17-year old girl with the phenotype of facioscapulohumeral muscular dystrophy experienced the onset of her symptoms in the neonatal period. The symptoms included muscle weakness, predominantly on upper limbs, scapulae alatae, craniofacial dysmorphia with ptosis and hypomimia. Analysis of the *FSHD1* locus was followed by next-generation sequencing (NGS) using a custom-designed neuromuscular gene panel. The results were verified by Sanger sequencing in both healthy parents and brothers. In vitro electrophysiological characterization of the variant was performed in order to determine its functional impact.

Results: *FSHD1* testing was negative. NGS revealed homozygous missense p.(Ala265Val) variant in the *SCN4A* gene (NM_000334.4):c.794C>T in the proband. Parents and both brothers were heterozygous for the variant. It is classified in ClinVar as a variant of unknown significance, predicted as deleterious by SIFT and disease-causing by MutationTaster. Population frequency in gnomAD ALL is 0,0018 %. The patch clamping of the "mutated" cells showed lower currents compared to the "wild type" cells.

Conclusions: Our results suggest that the *SCN4A* variant c.794C>T may explain the cause of muscular weakness in our proband and further broaden the spectrum of AR inheritance for *SCN4A*.

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P10.043.C Case report: The novel homozygous nebulin mutation c.18800T>C (p.Leu6267Pro) causes lethal arthrogryposis multiplex congenita

Naomi Baba[1], *Isolde Schreyer*[1], *Rosemarie Fröber*[2], *Bernhard Theis*[3], *Ekkehard Schleußner*[4], *Christian Andreas Hübner*[1], *Shoko Komatsuzaki*[1]

[1]*Institute of Human Genetics, University Hospital Jena, Jena, Germany,* [2]*Institute of Anatomy, Friedrich Schiller University Jena, Jena, Germany,* [3]*Section Pathology of the Institute of Forensic Medicine, University Hospital Jena, Jena, Germany,* [4]*Department of Obstetrics, University Hospital Jena, Jena, Germany.*

Introduction: Arthrogryposis multiplex congenita (AMC) is a clinically and genetically heterogeneous disease characterized by congenital joint contractures. Several severe fetal neuromuscular disorders are known to cause AMC. Case: We here report a consanguineous couple with a history of a male deceased newborn and a female stillbirth. In both siblings, AMC had been diagnosed by prenatal ultrasound. The male patient was born at the 37th week of gestation and died postpartum. His younger sister died in the 32nd week of gestation. Autopsies in both patients revealed hypoplastic lungs, flexion contractures of hands and fingers, myocardial hypertrophy and dysmorphic features including hypotrophic antihelix of ears, hypertelorism and high arched palate.

Results: After genetic counseling, an AMC gene panel was performed and revealed no disease-causing mutation. Exome sequencing allowed us to identify the novel homozygous variant c.18800T>C, p.(Leu6267Pro), rs184723737 in *NEB* in both patients. Both parents were heterozygous for this variant.

Discussion: *NEB* encodes nebulin, a giant cytoskeletal protein (773 kDa) expressed in skeletal muscle. Biallelic pathogenic variants in *NEB* cause nemaline myopathy, in which severities vary from prenatal lethality, severe early infantile neuromuscular disease to milder adult-onset phenotypes. Patients with severe nemaline myopathy show low set ears, high-arched palate, hypertelorism, which were observed in our patients. Because of the clinical findings of our patients, the evolutional conservation of p.Leu6267 and the co-segregation of the variant, we identify the novel c.18800T>C, p.(Leu6267Pro) variant as disease causing.

Conclusion: Our finding highlights the importance of whole exome sequencing in identifying rare genetic causes of AMC.

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P10.044.D Antigen presenting cells from PD patients exhibit an autoinflammatory cytokine profile

Camille Michaud[1,2], *Camberly Hernandez Paredes*[1,2], *Renaud Balthazard*[1,2], *Lovatiana Andriamboavonjy*[1,2], *Annie Laplante*[1], *Sébastien Audet*[1,2], *Martine Tétreault*[1,2], *Diana Matheoud*[1,2]

[1]*Centre de Recherche du CHUM, Montréal, QC, Canada,* [2]*Université de Montréal, Montréal, QC, Canada.*

Introduction: Elevated levels of pro-inflammatory cytokines have been shown in the serum of Parkinson's disease (PD) patients and mouse models suggesting that peripherally occurring inflammatory processes participate in PD pathogenesis. However, the cells responsible for this dysregulated production of cytokines, their exact expression profile and the consequences on T cell polarization remain unknown. Furthermore, PINK1, a PD-related gene, represses pro-inflammatory cytokine production mediated by the cGAS/STING pathway (an innate immune signaling pathway that detects cytosolic DNA). PINK1 is also a major repressor of mitochondrial antigen presentation (MitAP), a process dependent on mitochondrial-derived vesicle (MDV) formation and involved in mitochondrial autoimmune responses and PD pathogenesis. We hypothesize that antigen presenting cells (APCs) exhibit a dysregulated cytokine profile in PD that skew T cell polarization.

Materials and methods: Monocyte-derived dendritic cells (MDDCs) from PD patients and sex/age matched healthy individuals were generated from PBMCs to characterize their cytokine expression profile after LPS or bacterial stimulation using RT-qPCR and ELISA.

Results: We report that MDDCs from PD patients present a dysregulated cytokine profile. Moreover, only a subset of cytokines is altered which promotes the T cell polarization towards autoimmune-related Th17 cells.

Conclusions: Our results indicate that the dysregulated inflammation observed in PD patients affects APCs by inducing the production of pro-Th17 cytokines. These data strongly support the hypothesis that autoimmune mechanisms are implicated in PD. This project should allow us to better understand the role of autoimmunity in PD and to identify new biomarkers. Acknowledgements: CIHR, FRQS, Courtois foundation and Parkinson Canada

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P10.047.C Prediction of Parkinson's disease risk based on genetic profile and established risk factors

Paraskevi Chairta[1], *Andreas Hadjisavvas*[2,3], *Maria A. Loizidou*[2,3], *Kristia Yiangou*[2,3], *Andrea N. Georgiou*[4], *Christiana A. Demetriou*[5], *Yiolanda P. Christou*[1], *Marios Pantziaris*[1,2], *Kyriaki Michailidou*[2,6], *Eleni Zamba-Papanicolaou*[1,2]

[1]*Neurology Clinics, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus,* [2]*Cyprus School of Molecular Medicine, Nicosia, Cyprus,* [3]*Department of Electron Microscopy and Molecular Pathology, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus,* [4]*Department of Hygiene and Epidemiology, School of Medicine, University of Ioannina, Ioannina, Greece,* [5]*Department of Primary Care and Population Health, University of Nicosia Medical School, Nicosia, Cyprus,* [6]*Biostatistics Unit, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus.*

Introduction: Parkinson's disease (PD) is a neurodegenerative disorder and literature suggests that genetics and lifestyle/environmental factors may play a key role in the triggering of the disease. Polygenic risk scores (PRS) provide an aggregate score of variants that have been shown to be associated with a specific disease in GWAS. This study aimed to evaluate the predictive performance of a 12-SNPs PRS in combination with already established PD-environmental/lifestyle factors.

Materials and Methods: Genotypic, demographic and lifestyle data on 235 PD-patients and 464 healthy controls were obtained from a case-control study previously carried out in the Cypriot population. Logistic-regression analysis was used to assess the association of PRS with PD-status and each risk factor with PD-status. Stepwise-regression analysis was used to select the best predictive-model for PD combining environmental/lifestyle and genetic factors.

Results: The 12-SNPs PRS was significantly increased in PD cases compared to controls OR(95%CI). Logistic-regression analyses showed that age, head injury, family history and depression were positively associated with PD risk, while BMI was inversely associated with PD risk. Stepwise-regression suggested that a model which contains eight-independent factors including PRS is most predictive of PD.

Conclusions: These results suggest an association between both genetic and environmental factors and PD, and highlight the potential for the use of PRS in combination with the classical risk factors for risk prediction of PD. Further investigation with a larger cohort and a PRS with additional variants may increase the statistical-power and confirm that the combination of these factors could be used for prediction.

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P10.048.D Identification of a novel mutation of PIEZO2 gene as a cause of Distal Arthogryposis on a child: a case report

Javier Torres, *Fernando Macho, María L. Quintanilla, Esther Simarro, María C. Carrascosa, Laura Navarro*

Hospital General Universitario de Albacete, Albacete, Spain.

Distal Arthrogryposis with Impaired Proprioception and Touch (DAIPT, MIM #617146) is a rare disease characterized by a partial loss of mechanosensation. It can result in ataxia, muscle weakness, dysmetria and other alterations on walk and movement. It is recessively inherited and caused by homozygous or compound heterozygous mutations in *PIEZO2* gene. This gene codes a large transmembrane protein having a role in the conversion of mechanical stimuli into currents in somatosensory neurons.

A 4 year old child was referred with symptoms compatible with the disease, including (among other symptoms) impaired proprioception, congenital malformation of the Central Nervous System and hypotony.

A first study found variants in genes *SCN9A* and *SPTAN1*, both related with neurological pathologies, but the subsequent segregation study discarded them as the cause of the pathology. A further analysis found a novel mutation in *PIEZO2* gene (Cr. 18p11, c.3539_3572delAGTATTTCATCTGCATTGGCATCCCACCTGCTCC, p.(Gln 1180Leufs*19)),combined with a second mutation (c.18885G>T, p.(Glu*)) found in less than 0.001% of population. Our in-silico study of the variant's effect on the protein showed that these alterations cause an important truncation of the protein. Also, the segregation study demonstrated that each mutation was inherited from a different parent. All together, we concluded that the combination of both variants is the most likely cause of the disease in the child.

Although there is no treatment at the moment for this pathology, this knowledge can be useful in genetic counseling in this family and in the case of further research could provide new treatments for patients.

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P10.049.A <Frequency of carrier of AAGGG repeat expansions in *RFC1* gene in patients with sensorial peripheral neuropathy.>

María Fenollar-Cortés[1,2], *Carmen Cotarelo-Pérez*[1,2], *Raluca Oancea-Ionescu*[1,2], *Alejandro Horga*[3,2], *Antonio Guerrero-Sola*[3,2], *Lucía Galán-Dávila*[3,2], *Clara Herrero-Forte*[1,2]

[1]*Unidad de Genética Clínica. Servicio de Análisis Clínicos. Instituto de Medicina del Laboratorio. Hospital Clínico San Carlos, Madrid, Spain,* [2]*Instituto de Investigación Clínico San Carlos (IdISSC). Hospital Clínico San Carlos, Madrid, Spain,* [3]*Unidad de Enfermedades Neuromusculares, Servicio de Neurología, Hospital Clínico San Carlos, Madrid, Spain.*

Introduction: Biallellic intronic AAGGG expansion in the *RFC1* gene cause cerebellar ataxia, neuropathy, and vestibular areflexia syndrome (CANVAS). It has been estimated that allele frequency for the heterozygous AAGGG expansion in the general population is between 2-4%. In this study, we aimed to evaluate the carrier frequency of AAGGG and AAAGG repeat expansions in *RFC1* in a cohort of patients with idiopathic sensory peripheral neuropathy.

Materials and Methods: Conventional PCR and repeat-primed PCR (as described by Cortese *et al*) in the *RFC1* gene were analyzed in 95 patients with sensory peripheral neuropathy with or without additional clinical features. Patients with previously confirmed CANVAS were excluded.

Results: Sixteen patients (16.8% of all tested patients) carried a heterozygous, pathogenic AAGGG repeat expansion, and five patients (5.3%) carried a heterozygous, uncertain pathogenicity AAAGG repeat expansion. Overall, 22.1% of patients carried a heterozygous AAGGG or AAAGG repeat expansion in *RFC1*.

Conclusions: In our cohort, the carrier frequency of AAGGG repeat expansions in patients with sensory neuropathy was higher than the expected from general population estimates. Although previously reported heterozygous carriers are asymptomatic, it has been recently described that the carrier frequency of AAGGG repeat expansions is up to 21.2% among patients with late-onset ataxia. Further studies are needed to confirm the observed high carrier frequency of heterozygous *RFC1* repeat expansions in patients with sensory neuropathy and to improve the understanding of its clinical implications.

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P10.050.B Rhabdomyolysis-myalgia-syndrome in a family-clinical, molecular and therapy features

Dorothea Wand

Institut für Medizinische Genetik und Pathologie, Basel, Switzerland.

Rhabdomyolysis (RM) is defined as an acute elevation of plasma creatine kinase (CK>10 000 U/L) with painful muscles. The most frequent cause are toxic, trauma or external physical activity. The common cause of genetic muscle disorders with RM and myalgia are metabolic myopathies (e.g. McArdle, muscle glycolysis). Mutation in the ryanoidine receptor 1 gene *(RYR1)* is suggest to the common cause. We present the medical history, clinical, ancillary findings and an important therapy of two brothers and her family with hyperCKemia, RM and myalgia. In the metabolic myopathic panel (64gene) was identified a heterozygous pathogenic mutation c.7360C>T in the *RYR1-* gene. Mutations in this gene are causal for various congenital myopathies including maligne hyperthermia and susceptibility RM. A possible prophylactic treatment for RYR1- related RM are discussed Dantrolene. It is a muscle relaxant that selectively blocks the RYR1 channel. In our family was diagnosed a muscle ryanodine receptor - rhabdomyolysis- myalgia- syndrome.

D. Wand: None.

P10.051.C Identification by NGS of a novel mutation in the *TTN* gene in a Moroccan patient with Salih myopathy

Youssef El Kadiri[1,2], *Ilham Ratbi*[1], *Abdellatif Labiad*[3], *Jaber Lyahyai*[1], *Abdelaziz Sefiani*[1,2]

[1]*Research Team in Genomics and Molecular Epidemiology of Genetic Diseases, Genomic Center of Human Pathologies. Faculty of Medicine and Pharmacy, Mohammed V University, Rabat, Morocco,* [2]*Department of Medical Genetics, National Institute of Health, Rabat, Morocco,* [3]*Pediatric practice, Meknes, Morocco.*

Introduction: Titin is a giant protein encoded by the TTN gene with 364 exons. It is considered a principal regulator of the contractile behavior of striated muscle. Several pathologies are caused by pathogenic variants in the TTN gene and constitute a heterogeneous group of diseases. Salih myopathy, also known as early-onset myopathy with fatal cardiomyopathy (EOMFC), is an autosomal recessive congenital titinopathy affecting both skeletal and cardiac muscles. Herein, we describe clinical and molecular findings of a female patient analyzed by clinical exome sequencing (CES).

Patient and Methods: In this study, we report the case of a consanguineous Moroccan child aged 2 years and 5 months at the time of her genetic assessment. She was referred to our medical genetic center for clinical features of a congenital myopathy associated with dilated cardiomyopathy (DCM), in favor of Salih myopathy. CES was performed to screen among the neuromuscular genes, the disease-causing mutation more precisely.

Results: Bioinformatics analysis identified a novel homozygous truncating mutation NM_001267550.2; LRG391_t1:c.106541delA, p.(Asp35514ValfsTer32) in exon 361 of the TTN gene. It has never been reported previously in public human databases. Prediction websites considered this mutation as a pathogenic variant. Sanger sequencing confirmed it in the affected patient and showed that both parents were heterozygous.

Conclusions: Nowadays, the application of next-generation sequencing technology in Moroccan medical practice is becoming possible. Molecular diagnosis of clinical and genetic heterogeneous diseases such as titinopathies, allows the detection of a specific variant in the causative gene and expands the mutational spectrum of Moroccan patients.

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P10.052.D Pathogenic *SAMD9L* variants: Differential diagnosis of CMT and potential pitfall in trio-exome analysis

Katja Eggermann[1], *G. Christoph Korenke*[2], *Ingo Kurth*[1], *Matthias Begemann*[1], *Daniela Dey*[1], *Cordula Knopp*[1]

[1]*Institute of Human Genetics RWTH University Hospital, Aachen, Germany,* [2]*Klinikum Oldenburg, Oldenburg, Germany.*

Introduction: Autosomal dominant mutations in *SAMD9L* cause Ataxia-pancytopenia (ATXPC-) syndrome characterized by a variable hematological (pancytopenia, predisposition to bone marrow failure, myelodysplasia, and myeloid leukemia) and neurological (cerebellar ataxia and atrophy, nystagmus, mild pyramidal signs and white matter abnormalities) phenotype. Association of peripheral neuropathy has rarely been described and a pathogenic *SAMD9L* variant causing Charcot-Marie-Tooth (CMT) disease has been reported only once. Case Report: A 37-year-old female presented with demyelinating neuropathy and axonal involvement. High arched feet and brisk reflexes at the knees were reported. The 14-year-old daughter was similarly affected and had an unremarkable brain MRI at age 10 years. There was no history of hematological abnormalities.

Results: Trio whole-exome-sequencing was performed in the mother and her unaffected parents without conclusive results. Inclusion of the daughter led to the identification of a previously reported likely pathogenic variant in the *SAMD9L* gene (NM_1512703.4:c.2956C>T; p.(Arg986Cys)). Re-analysis of the mother's data showed low-grade mosaicism for this variant, which was below the threshold of the analysis pipeline.

Conclusions: 1) The clinical picture of our patients confirms that mutations in *SAMD9L* may present as demyelinating neuropathy and need to be considered as differential diagnosis of CMT. This is of particular importance as there is a need for surveillance of potential hematologic manifestations in patients with *SAMD9L* mutations. 2) Low-grade mosaicism of de-novo mutations may be missed in trio-exome sequencing approaches, which should carefully be taken into account.

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P10.053.A Differential diagnosis of SMA and congenital myopathies using the targeted MPS panel

Polina Chausova, *Oksana Ryzhkova, Elena Dadali, Victoria Zabnenkova, Aleksandr Polyakov*

Research Centre for Medical Genetics named after academician N.P. Bochkov, Moscow, Russian Federation.

Introduction: Spinal muscular atrophy (SMA) is the most common hereditary disorder, one of the key symptoms of which is hypotension since birth or early childhood. The basic method for diagnosing SMA is the study of extended deletions in the *SMN1* gene with an estimate of the number of copies of the pseudogene using the MLPA method. For some patients (about 50%), it is not possible to confirm the disease by molecular genetic methods, which indicates the presence of another pathology in this group of patients.

Materials and Methods: Using the MPS method, using the congenital muscular dystrophies target panel, a sample of unrelated patients with a referring SMA diagnosis without deletions in the *SMN1* gene (78 patients aged 0 to 2) was studied.

Results: Pathogenic and probably pathogenic variants were detected in the *LAMA2* gene in 3 probands, in two in the ACTA1 and *MTM1* genes, single patients had mutations in the *COL12A1, LMNA, LMOD, COL6A1, COL6A3* genes. In addition, in 8 patients in the genes *LAMA2, COL5A1, CCDC78, COL12A1, MTMR14*, variants of uncertain clinical significance were identified, which are the most promising from the point of view of converting them into probably pathogenic ones after additional studies.

Conclusion: It has been shown that, in a sample of SMA patients without an extended deletion of exon 7 in the *SMN1* gene, in more than 15.4% of cases, the molecular cause of the disease is mutations in the genes responsible for the development of congenital myopathies.

P. Chausova: None. **O. Ryzhkova:** None. **E. Dadali:** None. **V. Zabnenkova:** None. **A. Polyakov:** None.

P10.054.B Role of SMN in the nucleolar reorganization after DNA repair

Shaqraa musawi[1,2], *Lise-Marie Donnio*[1], *Giuseppina Giglia Mari*[1]

[1]*Institut NeuroMyoGène, LYON, France,* [2]*Department of medical laboratory technology, Jazan, Saudi Arabia.*

Introduction: Most cellular transcriptional activity is carried out by the RNA polymerase I (RNAP1), which transcribe ribosomal DNA (rDNA) into ribosomal RNA (rRNA) needed in the ribosome biogenesis. During DNA Repair of UV-lesions, rDNA/RNAP1 are both reorganized within the nucleolus, namely, they migrate at the periphery of the nucleolus during DNA repair reactions and come back within the nucleolus after DNA repair completion. The proteins and exact mechanism behind these movements remain not understood.

Materials and methods: We employed various cellular and molecular biology methods, combined with confocal microscope procedures on knockdown Survival Motor Neurons (SMN) cells, and on cells of patients affected by Spinal Muscular Atrophy (SMA), to investigate whether SMN may play a role in the nucleolar reorganization during DNA repair.

Results: Our results clearly demonstrate the involvement of the SMN in the nucleolar reorganization after DNA repair. In fact, in the absence of SMN, RNAP1 and Fibrillarin (FBL), an essential nucleolar protein, do not return inside the nucleolus after DNA repair completion while transcription is fully restored. Interestingly, SMN is strongly, accumulating within the nucleolus after DNA repair and concomitantly, we revealed a strong interaction between SMN and FBL after the completion of DNA repair.

Conclusions: Our findings identify a new function for SMN in the re-establishment of the proper nucleolar organization after DNA repair. These results may have important implications in identifying the biological origins of spinal muscular atrophy disorder.

S. musawi: None. **L. Donnio:** None. **G. Mari:** None.

P10.055.C Detection of multiple variants in complex family trio with neurodegenerative diseases

Sebastien Audet[1,2], *Eric Samarut*[1], *Martine Tetreault*[1,2]

[1]*CHUM Research Center, Montreal, QC, Canada,* [2]*Department of Neurosciences, University of Montreal, Montreal, QC, Canada.*

After thorough yet inconclusive clinical genetic testing to uncover the cause of neurodegenerative symptoms in a father and his daughter, our lab was tasked to do a more in-depth genomic investigation of the family. Previous assessment of phenotypes by medical professional suggested a probable shared pathology, albeit with an anticipation effect, since the daughter exhibited worsened and earlier onset of disease. A repeat expansion variant was therefore suspected.

We extracted DNA from the saliva of the two patients and the mother. Whole-Genome Sequencing (WGS) was performed. We used bioinformatics tools for alignment, quality control, and calling of single nucleotide polymorphism, copy number variants and repeat expansions. Long-read and Sanger sequencing, as well as qPCR, were performed to validate the potential pathogenicity of the candidate variants.

Although both patients underwent multiple gene panels for neurologic and neuromuscular diseases prior to WGS, we detected a non-synonymous (R499H) mutation in the *SPAST* gene of the daughter. A diagnosis could then be made as the variant was previously reported as probably pathogenic for spastic paraplegia type 4. On the other hand, the father presented a bi-allelic repeat expansion in *RFC1* locus, presumably causing CANVAS disorder, while the daughter carried only one expanded allele. Sanger and long-read sequencing respectively confirmed the variants.

While the father had been previously tested for *SPAST*, the assumption of a common genetic cause prohibited an early diagnosis of the daughter for spastic paraplegia. These results highlight the importance of unbiased genetic analyses in clinical settings.

CIHR, FRQS, National Ataxia Foundation

S. Audet: None. **E. Samarut:** None. **M. Tetreault:** None.

P10.056.D Phenotype in Associated SMA mutations - experience of 5 years

Ramona Babici[1], *Maria Tonu*[1], *Mihaela Danila*[1], *Doina Ursan*[1], *Roxana Popescu*[2,3], *Lacramioara Butnariu*[2,3], *Monica Panzaru*[2,3], *Eusebiu Vlad Gorduza*[1,3], *Cristina Rusu*[2,3]

[1]*"Cuza Voda" Maternity Hospital, Iasi, Romania,* [2]*"Saint Mary" Emergency Children's Hospital - Regional Medical Genetics Centre, Iasi, Romania,* [3]*"Grigore T. Popa" University of Medicine and Pharmacy - Department of Medical Genetics, Iasi, Romania.*

Introduction: Spinal Muscular Atrophy (SMA) is an autosomal recessive neuromuscular disorder due to homozygous loss of

function in SMN1 gene. The phenotypic severity of the disease may be influenced by several modifying factors: SMN2 gene and other genes like: BIRC1, NAIP, RAD17, GTF2H2, SERF1A, N-Cadherin-like. Our aim was to describe the phenotype-genotype correlation in SMA patients who associate mutations in modifying genes.

Methods: We have analyzed 23 patients from North-Eastern Romania diagnosed clinically with SMA in Iasi Regional Medical Genetics Center in the last 5 years. All patients were investigated by MLPA using the P021-A1 SMA probe mix kit. This is a molecular genetic screening test to identify variations in the number of copies (deletions / amplifications) of 37 sequences in the 5q13 region. The main genes targeted are SMN1, SMN2, and other genes from this region that may influence the phenotype.

Results: From 23 patients clinically diagnosed with SMA, 22 had homozygous deletions of exons 7 and 8 of the SMN1 gene, while 1 patient had a normal number of copies of exon 8 in SMN1. Only one patient in this group had heterozygous deletion in the SMN2 gene, the rest presented 2-4 copies of the SMN2 gene. 10 patients associated changes in at least one of the genes: NAIP, GTF2H2, SERF1B. Nine of them had severe SMA subtypes (I and II).

Conclusion: The presence of homozygous or heterozygous deletions identified in the NAIP, GTF2H2, SERF1B genes may suggest a more severe phenotype.

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P10.057.A Genomic Precision Diagnosis of a Genetically Complex Case of Spinocerebellar Ataxia

*Saba Habibollah[1], **Lova Satyanarayana Matsa**[2]*

[1]Iranian Hospital, Dubai, United Arab Emirates, [2]Igenomix FZ LLC, Dubai Healthcare City, Dubai, United Arab Emirates.

A 26-year-old male presented with progressive ataxia for the past 3 years, with muscle weakness, exercise intolerance, and slurred speech. Brain CT showed cerebellar atrophy and expression of prominent cisterns, cerebellar and vermian folia, given clove leaf-like appearance consistent with hereditary cerebellar degeneration. He was born to a healthy consanguineous couple. He has two healthy siblings while one of his maternal cousins suffers from severe tremors and ataxia preceded by fever. He was found to be negative for the most common trinucleotide repeats expansions in spinocerebellar ataxia genes. Through whole-exome sequencing, he was found to carry a homozygous frameshift variant in exon 51 of the *SYNE1* gene (NM_182961.4:c.7557delC). Biallelic *SYNE1* mutations are known to cause autosomal recessive spinocerebellar ataxia-8 (SCAR8), which is a slowly progressive neurodegenerative disorder characterized by gait ataxia with cerebellar signs, such as nystagmus and dysarthria. The age at onset is highly variable, usually in the second decade. The clinical profile of the index is more appropriate with the SCAR8 phenotype, and the family history also suggests an autosomal recessive disorder. Additionally, a heterozygous donor splice site variant in intron 5 of the *MME* gene (NM_000902.3:c.439+1G>A) was detected. Heterozygous mutations in the *MME* gene cause autosomal dominant spinocerebellar ataxia-43 (SCA43), which is a slowly progressive neurologic disorder characterized by adult-onset gait, limb ataxia, and often associated with peripheral neuropathy. Both these identified mutations are novel and classified as likely pathogenic as per ACMG guidelines. Segregation analysis is required to confirm their final significance, which is under process.

S. Habibollah: None. **L.S. Matsa:** None.

P10.058.B Deep analysis of splicing variants in DMD gene

***Mikhail Yu Skoblov**, Ksenia Davydenko, Alexandra Filatova*

Research Center for Medical Genetics, Moscow, Russian Federation.

Introduction: Mutations in the DMD gene, which encodes the protein dystrophin, lead to severe Duchenne muscular dystrophy and milder Becker muscular dystrophy. The type of mutation and the current molecular mechanism determine the development and progression of the disease. Splicing variants are one of such types of mutations that are not easy to reveal and predict its effect, and functional analysis is required for their validation.

Materials and methods: SpliceAI (Illumina, USA) and MaxEntScan (MIT, USA) were used to predict the splicing effect of SNV. To evaluate the functional effect of SNVs, the minigene expression assay was performed in HEK293T cells. SNVs were introduced into expression plasmids by site-directed mutagenesis.

Results: We carried out deep in silico mutagenesis of the entire DMD gene sequence to search for all possible splicing variants with SpliceAI. About 8000 candidate splicing variants were found in introns and in exons of the gene. We overlapped our splicing SNVs with variants from the HGMD database and found 90 exonic variants and more than 300 SNVs in introns. Only for a few of them the effect on splicing been confirmed experimentally. We created 20 minigene systems with coding exons of DMD and performed the functional analysis for more than 30 SNV (missense, nonsense and synonymous). For each variant it was demonstrated the molecular mechanism of splicing disruption.

Conclusion: Our analysis revealed all possible splicing variants in the DMD gene. Functional analysis with minigene systems confirmed the existence of hundreds of misannotated variants in HGMD.

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P11 Multiple Malformation/Anomalies Syndromes

P11.001.C 10q26 deletion syndrome: a French cohort study

***Hugo Thorn**[1], Sylvie Odent[2,3], Jonathan Levy[4,5], Anne-Claude Tabet[4,6], Julien Thevenon[7,8], Cedric Le Caignec[9], Elise Schaefer[10], Thierry Frebourg[11], Caroline Schluth-Bolard[12,13], Morgane Plutino[14], Salima El Chehadeh[10], Anais Philippe[10], Sophie Scheidecker[15], Nadege Calmels[15], Audrey Schalk[15], Alice Goldenberg[11], Anne-Marie Guerot[11], Nathalie Le Meur[16], Kevin Cassinari[16], Lyse Ruaud[4,5], Myriam Rachid[17], Louis Januel[12], Marie-Noëlle Bonnet-Dupeyron[12,18], Maryline Carneiro[19], Eric Bieth[20], Julie Plaisancie[20], Charles Coutton[21,22], Radu Harbuz[21], Klaus Dieterich[23,24], Gwenaël Nadeau[25], Gaelle Vieville[26], Melanie Fradin[27], Celine Poirsier[1], Marta Spodenkiewicz[1], Emilie Landais[28], Martine Doco-Fenzy[1,28,29]*

[1]Département de Génétique, CHU de Reims, Reims, France, [2]Service de Génétique, CHU de Rennes, Rennes, France, [3]UMR 6290 Université de Rennes, Rennes, France, [4]Département de Génétique, APHP Nord, Hôpital Robert Debré, Paris, France, [5]Université de Paris, UMR 1141 NEURODIDEROT, INSERM, Paris, France, [6]Département de neurosciences, Unité GHFC, Institut Pasteur, Paris, France, [7]Department of Genetics and Reproduction, Centre Hospitalier Universitaire de Grenoble, Grenoble, France, [8]CNRS UMR 5309, INSERM, U1209, Institute of Advanced Biosciences, Université Grenoble-Alpes CHU Grenoble, Grenoble, France, [9]Laboratoire de Cytogénétique, CHU de Toulouse, Toulouse, France, [10]Service de Génétique Médicale, Institut de génétique médicale d'Alsace (IGMA), Hôpitaux Universitaires de

Strasbourg, Strasbourg, France, [11]Service de Génétique Médicale, CHU de Rouen, Rouen, France, [12]Service de Génétique, Centre de Biologie et de Pathologie Est, Hospices Civils de Lyon, Bron, France, [13]Institut Neuromyogène, Equipe Métabolisme énergétique et développement neuronal, CNRS UMR 5310, INSERM U1217, Université Lyon 1, Lyon, France, [14]Service de Génétique Médicale, Hôpital de l'Archet II, CHU Nice, Nice, France, [15]Laboratoires de Diagnostic Génétique, Unité de Cytogénétique Chromosomique et Moléculaire, Institut de génétique médicale d'Alsace (IGMA), Hôpitaux Universitaires de Strasbourg, Strasbourg, France, [16]Laboratoire de Cytogénétique, Service de Génétique Médicale, CHU de Rouen, Rouen, France, [17]Laboratoire de Cytogénétique, Département de Génétique, APHP Nord, Hôpital Robert Debré, Paris, France, [18]Service de Cytogénétique, Centre Hospitalier de Valence, Valence, France, [19]Service de Neuropédiatrie, Hôpital Femme Mère Enfant, Hospices Civils de Lyon, Bron, France, [20]Département de Génétique Médicale, CHU de Toulouse, Toulouse, France, [21]CHU Grenoble Alpes, UM de Génétique Chromosomique, Grenoble, France, [22]Univ. Grenoble Alpes, INSERM U1209, CNRS UMR 5309, Institute for Advanced Biosciences, Team Genetics Epigenetics and Therapies of Infertility, Grenoble, France, [23]CHU Grenoble Alpes, UM de Génétique Clinique, Grenoble, France, [24]INSERM U1216 Grenoble Institut des Neurosciences, Cellular Myology and Pathology, Grenoble, France, [25]Laboratoire de Génétique Chromosomique, Centre Hospitalier Métropole Savoie, Chambery, France, [26]CHU Grenoble Alpes, UF de Génétique Chromosomique, Grenoble, France, [27]Service de Génétique CHU Rennes, Centre de Référence Anomalies du Développement, Rennes, France, [28]Laboratoire de Cytogénétique et Génétique Moléculaire, CHU Reims, Reims, France, [29]EA381 SFR CAP Santé Reims, Reims, France.

10q26 deletion syndrome (OMIM #609625) is a rare autosomal dominant genetic disorder with about 100 patients reported. Most cases are sporadic. Global development delay, short stature, microcephaly and typical facial appearance with triangular face, large forehead, low-set malformed ears, hypertelorism, prominent nose and a thin vermilion of the upper lip constitute the main clinical features. The clinical spectrum is very heterogeneous and neurobehavioral manifestations, deafness, limb malformations, cardiac and urogenital abnormalities can be associated. Thus, patients with 10q26 chromosomal deletion need multidisciplinary management strategies from birth. One of the main reasons for this heterogeneity is the variety of 10qter region chromosomal deletions summarized into the "10q26 deletion syndrome". Various studies proposed critical regions to explain the main phenotype (Yatzenko et al., 2009; Choucair et al., 2015; Lin S et al., 2016) or more specific features (Vera-Carbonell et al., 2015; Choucair et al., 2015). In addition, these studies proposed about 20 genes of interest such as *DOCK1* and *FGFR2* to explain the different clinical features observed. We report a French ACLF cohort of 35 patients from 9 centers presenting 10q26 complete or partial deletions (size: 64kb to 12.5Mb), complex chromosomal rearrangement and derivative chromosomes diagnosed using DNA-array, to bring a further insight of the genotype/phenotype correlation.

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P11.002.D Chromosome 13q deletions syndrome and clinical reflections in our case: a case report

***busra goksel tulgar**, fahrettin duymus, deniz esin, ebru marzioglu ozdemir, nadir kocak*

Selcuk University, Konya, Turkey.

Chromosome 13q deletion syndrome is a rare chromosomal disorder. The patient's clinic varies depending on the size and localization of the deletion. As far as chromosome 13 is known, it contains about 300 genes that synthesize active proteins. The disease creates a broad spectrum in affected individuals, including developmental delay, intellectual disability, low birth weight, skeletal abnormalities, and other congenital malformations. For these reasons, it is difficult to define a specific clinical phenotype in these patients. We aimed to present the deletions in the 13q region of our patient and their clinical implications in our case. Our case was a 4-year-old male patient at the time of his admission. Basically, we were consulted with the complaint of speech delay. Physical examination revealed a triangular face, broad and flat nasal bridge, micrognathia, large ears, short neck, undescended testis, growth retardation. There was retardation in the neuromotor stages of development. There is no consanguinity between his parents. Two Secundum ASDs were found on echocardiography. 13q deletion was detected in the chromosome analysis. In the microarray analysis performed on this, it was confirmed that there was a deletion in the chromosome 13q21.1q31.1 region. His parent's chromosome analysis is normal. Genetic consultation was given to the family of the patient and referred to the relevant departments. 13q deletion is a rare phenomenon and each patient should be evaluated individually. Unfortunately, there is no definitive treatment. It is typically not inherited. The loss of part of the chromosome occurs during gametogenesis, making it a de novo mutation. When inherited, it is usually caused by a mosaic or balanced translocation parent. As the genes in the deletion area are enlightened, patients can be diagnosed more easily.

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P11.003.A First case report of deletion 13q22.2q31.1-expanding phenotype spectrum of chromosome 13q deletion syndrome

***Mijana Kero**[1,2], Leona Morožin Pohovski[1], Adriana Bobinec[1,2], Nikolina Vidan Rogulj[1], Ana-Maria Meašić[1,2], Ljubica Boban[1,2], Ivona Sansović[1,2], Ingeborg Barišić[1,2]*

[1]Children's Hospital Zagreb, Zagreb, Croatia, [2]Department of Medical Genetics and Reproductive Health, Children's Hospital Zagreb, Scientific Centre of Excellence for Reproductive and Regenerative Medicine (CERRM), University of Zagreb School of Medicine, Zagreb, Croatia.

Introduction: Interstitial deletions 13q are rare and characterized by variable phenotype including psychomotor and growth delay, retinoblastoma, dysmorphic facial features, and various associated malformations, still without clear correlation between the 13q deletion intervals to distinct phenotypes.

Patients and Methods: Fifteen-month girl, born premature (29 weeks) with IUGR, birth weight 560 g, and hypoxia signs, spent 262 days in the intensive care unit due to recurrent sepsis, pancytopenia, and multiple complications of premature birth. She has a significant global developmental delay, microcephaly, mild hypertelorism, broad nasal bridge, nystagmus, partial corpus callosum dysgenesis, hypothyroidism, and bilateral inguinal hernias. The genomic DNA was isolated, and array CGH with Next Generation Sequencing (NGS) analysis, focusing on 1937 genes associated with clinical features, were done.

Results: Array CGH detected a *de novo* interstitial deletion 13q22.2q31.1 (6,13 Mb). NGS revealed a novel heterozygous missense variant (c.1235G>A, p.Cys412Tyr) in exon 12 of the *CUL5* gene (OMIM #601741), categorized as VUS according to ACMG/AMP classification, inherited from a healthy mother.

Conclusion: To our knowledge, this is the first patient described in the literature with defined breakpoints 13q22.2q31.1. Prematurity, pancytopenia, recurrent sepsis, hypothyroidism, and inguinal hernia have not been described so far in patients with 13q deletions. Clinical and molecular characterization of further patients with 13q deletions might contribute to its better genotype-phenotype correlation and understanding of pathogenesis. This study was supported by CERRM, Republic of Croatia, and by the EU through ERDF, under grant agreement No. KK.01.1.1.01.0008, project„Reproductive and Regenerative Medicine - Exploring New Platforms and Potentials".

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P11.005.C Neonatal diagnosis of 16p12.2 microdeletion syndrome

Pamela Paglia[1], *Mariateresa Falco*[2], *Dario Di Salvio*[3], *Piero Pignataro*[4], *Anna Rita Frascogna*[5], *Maria Grazia Corbo*[5], *Rita Genesio*[4], *Daniela Melis*[1]

[1]*Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", Section of Pediatrics, University of Salerno, Baronissi (SA), Italy,* [2]*Clinical Pediatrics, University Hospital "San Giovanni di Dio e Ruggi D'Aragona", Salerno, Italy,* [3]*Department of Translational Medical Sciences, Section of Pediatrics, University of Naples "Federico II", Napoli, Italy,* [4]*Department of Molecular Medicine and Medical Biotechnology, University of Naples "Federico II", Napoli, Italy,* [5]*Department of Maternal-Infant, Neonatalogy and Intensive Care Unit, University Hospital "San Giovanni di Dio e Ruggi D'Aragona", Salerno, Italy.*

Introduction: 16p12.2 microdeletion syndrome is characterized by minor facial abnormalities, developmental delay, behaviour disorders and other variable systemic features, with incomplete penetrance. It is usually diagnosed in children with neurobehavioral disorders; diagnosis in neonatal period has been described in only few patients.Clinical report. We report a newborn of healthy nonconsanguineous parents, born after a pregnancy complicated with polyhydramnios, at 36 weeks, by urgent cesarean delivery. Birth weight was 3,200 g (75-90th), length 43 cm (<3th), and OFC 34 cm (75th). At birth, he showed generalized cyanosis, hypotonia and poor respiratory activity which required non-invasive ventilation and admission in Intensive Care Unit. Poor sucking was also present. Physical examination showed plagiocephaly, bilateral temporal depression, hypertelorism, downslanting palpebral fissures, low-set ears, depressed nasal root, bulbous nasal tip, anteverted nostrils, retrognathy with chin dimple, short neck, sacral dimple with hypertrichosis. Brain ultrasound revealed hyperechoic lobulated areas in the left ventricle with extension into the subcortical area. Echocardiography showed a slight acceleration of the flow on the pulmonary branches. Audiologic evaluation, electroencephalogram and abdominal ultrasonography showed normal data.Array-comparative genome hybridization (array-CGH) showed a 478 Kb pathogenetic deletion in 16p12.2 region (critical region for 16p12.2 microdeletion syndrome), a deletion in the Xp22.33/Yp11.32 region of 13.5 Kb and a duplication in the Xq12 region of 671 Kb, containing part of the AR gene.

Conclusion: Array-CGH in neonate with hypotonia, feeding difficulties, short-length and craniofacial dysmorphisms could allow early diagnosis of microdeletion syndromes. This case could expand the clinical spectrum of 16p12.2 patients.

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P11.006.D Clinical findings in 22q11.2 microdeletion syndrome: case series

Sule Altıner, *Timur Tuncalı, Nüket Yürür Kutlay, Halil G. Karabulut, Hatice Ilgın Ruhi*

Ankara University, School of Medicine, Department of Medical Genetics, Ankara, Turkey.

Introduction: 22q11.2 microdeletion syndrome is the most common microdeletion syndrome with an incidence of 1 in 4,000 live births. The syndrome is caused by a 1.5 to 3.0-Mb deletion in q11.2 region of chromosome 22. Although phenotypic findings are highly variable, immune deficiency, congenital cardiac anomalies, speech delay and palatal anomalies are the most common.

Materials and Methods: A retrospective study investigating medical records of 22q11.2 microdeletion cases whose deletion was confirmed with FISH assay in Ankara University School of Medicine, Department of Medical Genetics between 1999-2020.

Results: A total of 28 patients (male, n = 22 and female, n = 6) were enrolled in this study. Mean age of the patients was 4,6 years at the diagnosis (median:0,675, range:0-33). The most common clinical findings were immunodeficiency/recurrent infections (75%, 21/28), congenital cardiac defects (75%, 21/28) and hypocalcemia (57,1%, 16/28). Also, typical dysmorphic features were noted in 75% (21/28) of patients. Learning difficulties, developmental delay and urogenital defects were subsequent common findings. Co-occurrence of psychiatric findings with dysmorphic features was the common cause of referral in adult patient group. Family study could be performed in 10 cases and 10% of them were found to be familial.

Conclusion: The results of this study are compatible with current literature. The frequency of psychiatric, neurologic and skeletal findings may increase with follow-up data of current patients. Further detailed multidisciplinary studies will better elucidate 22q11.2 microdeletion syndrome.

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P11.007.A Interstitial deletion of 2q32.3q33.3: Two case reports of SATB2-Associated-Syndrome and Immune System alterations

Joana Adelaide Catanho[1], *Ana Isabel Cordeiro*[2], *Teresa Kay*[1], *Inês Carvalho*[1]

[1]*Serviço de Genética Médica, Hospital Dona Estefânia, Centro Hospitalar e Universitário de Lisboa Central, Lisboa, Portugal,* [2]*Unidade de Imunodeficiências Primárias, Hospital Dona Estefânia, Centro Hospitalar e Universitário de Lisboa Central, Lisboa, Portugal.*

Introduction: Interstitial deletions of the long arm of chromosome 2 involving the 2q32q33 region encompass SATB2 gene. Its

haploinsuffuciency results in SATB2-Associated-Syndrome (SAS), characterized by neurodevelopment disorders, behavioral issues, palatal abnormalities and facial dysmorphism. Within this region, genes involved in Immune Disorders can be identified, among them, *CTLA4*. *CTLA4* haploinsufficiency is associated with autoimmune cytopenia, hypogammaglobulinemia, recurrent infections, diarrhea or inflammatory bowel disease.

Clinical Data: P1 is an 11-year-old boy, born at 36 weeks of gestation with cleft palate, micrognathia, left inguinal hernia and interatrial communication (IAC). He has psychomotor delay, hypotonia, hyperactivity, aggressiveness, epileptogenic activity, severe intellectual disability (ID), autoimmune thrombocytopenia, neutropenia and hemolytic anemia diagnosed at age 3. P2 is a 5-year-old boy, born at 38 weeks, with cleft palate, micrognathia, and IAC. He has severe psychomotor delay, behavior abnormalities, failure to thrive (at 14-months was placed with percutaneous endoscopic gastrostomy), epilepsy and recurrent infections.

Methods and Results: P1 microarray analysis identified a 12.2 Mb heterozygous deletion involving the interstitial chromosome region 2q32.3q33.3. P2 carried a 10.62 Mb heterozygous deletion involving the interstitial chromosome region 2q33.1q33.3. Both deletions encompass *SATB2* and *CTLA4* genes.

Conclusions: We believe the haploinsufficiency of both genes *SATB2* and *CTLA4*, by 2q32q33.3 microdeletion, might explain the phenotype of these patients. This report brings to our attention that 2q32q33.3 microdeletion can be associated with immune alterations. Recognition of this clinical signs and symptoms is of the most importance for patients' early referral for Immune Disorder Specialist.

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P11.008.B COLEC10 and 3MC syndrome: expanding the genotypic and phenotypic spectrum of a very rare disease

Martina Migliorero[1], *Silvia Kalantari*[2], *Valeria Bracciamà*[2], *Monica Sorbini*[1], *Francesca Arruga*[1], *Licia Peruzzi*[3], *Elisa Biamino*[4], *Antonio Amoroso*[1,2], *Tiziana Vaisitti*[1], *Silvia Deaglio*[1,2]

[1]*Department of Medical Sciences, University of Turin, Turin, Italy,* [2]*Immunogenetics and Transplant Biology Service, Città della Salute e della Scienza University Hospital, Turin, Italy,* [3]*Pediatric Nephrology Dialysis and Transplantation Unit, Città della Salute e della Scienza University Hospital, Turin, Italy,* [4]*Department of Pediatrics, AOU Città della Salute e della Scienza di Torino, University of Torino, Turin, Italy.*

Introduction: 3MC syndrome is an autosomal recessive disorder encompassing a variable spectrum of abnormalities, among which facial dysmorphisms are characteristic. Mutations in genes which encode proteins involved in the lectin complement pathway *MASP1*, *COLEC11* and recently *COLEC10* have been identified in patients with 3MC syndrome, supporting their role during human development. We present a 5 years old patient with typical 3MC phenotypic characteristics, including blepharophimosis, telecanthus, high arched eyebrows, fifth finger clinodactyly and horseshoe kidneys. The diagnosis was confirmed by sequencing of *COLEC10* gene and the putative pathogenic variant was functionally validated through in vitro assays.

Materials and Methods: *COLEC10* gene was analyzed through Sanger sequencing. The variant was introduced by a site-specific mutagenesis approach into a plasmid encoding wild-type human CL-L1. HeLa cells were transfected with the mutated or wild-type plasmid and culture supernatant evaluated in a migration assay.

Results: A homozygous frameshift variant c.807_810delCTGT;p.(Cys270Serfs*33) was identified in the patient. Segregation studies confirmed the parents' carrier status for the variant. Functionally, the variant affects the chemo-attractive feature of CL-L1, as HeLa cells are less sensitive to the mutant protein compared to the WT one, resulting in a reduced migratory response.

Conclusions: We report a patient affected by 3MC syndrome who, besides typical phenotypic signs, presents a patent ductus arteriosus, never described in association to *COLEC10* before. The variant causative role was functionally confirmed in an in vitro assay, where the mutated protein failed to act as a chemo-attractant. We thus provide further evidence for CL-L1 role during embryonic development.

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P11.009.C AKT3 variant in a patient with macrocephaly

Florina Victoria Nazarie[1], *Simona Bucerzan*[1,2], *Monica Mager*[3,2], *Diana Miclea*[1,2]

[1]*Genetic Department, Emergency Hospital for Children, Cluj-Napoca, Romania,* [2]*Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania,* [3]*Pediatric Neurology Department,Emergency Hospital for Children, Cluj-Napoca, Romania.*

Introduction: Megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome 2 (MPPH2) is an overgrowth syndrome comprising megalencephaly, hydrocephalus and polymicrogyria; polydactyly may also be seen. Activating mutations in AKT3 gene are a rare cause of megalencephaly. AKT3 is one of 3 closely related serine/threonine-protein kinases (AKT1,AKT2 and AKT3) which regulate processes including metabolism, proliferation, cell survival, growth and angiogenesis.

Materials and methods: We present a case of a four month old girl, who was referred to the genetic department because of macrocrania (50cm, Z score = +7.44 SDS, >p98), frontal bossing, generalised hypotonia, delay in motor acquisitions. Clinical exam revealed large anterior fontanelle 4x6cm and blue sclerae. Transfontanellle ultrasound was performed and no hydrocephalus was found. Multiple diagnoses were discussed: Sotos Syndrome, Glutaric aciduria type I, Megalencefaly-Capillary Malformation-Polymicrogiria Syndrome. Multiplex Ligation Probe Amplification (MLPA) with Probemix P245-B1 Microdeletion Syndromes was done for Sotos syndrome, but came back with no modification. The level of urinary glutamic acid was also normal. Whole exome sequencing (WES) was proposed and performed on Illumina HiSeq platform.

Results: WES revealed a likely pathogenic variant in AKT3 gene: exon4:c.250G>A:p.Glu84Lys (hg38), located in a functional region, "PH" AKT3_HUMAN domain.

Conclusions: Mutations of the AKT3 gene have been reported in a few individuals with brain malformations, activating variants are associated with diffuse megalencephaly with intellectual disability and/or autism spectrum disorder. Early diagnose can provide the appropriate management of the case and maybe find a suitable drug among inhibitors of PI3K-AKT-MTOR pathway.

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P11.010.D Identification and characterisation of two novel mutations in the MAN2B1 gene in middle-aged siblings

Márta Szegedi[1], *Gábor Rácz*[2], *Ágnes Palásti*[1], *Zoltán Grosz*[1], *Mária Judit Molnár*[1]

[1]*Semmelweis University, Institute of Genomic Medicine and Rare Disorders, Budapest, Hungary,* [2]*University of Szeged, Department of Pediatrics, Szeged, Hungary.*

Introduction: Alpha-mannosidosis is a rare inherited disorder caused by mutations in the MAN2B1 gene that encodes the lysosomal alpha-mannosidase. Our aim was to describe the phenotypes of a middle-aged siblings with alpha-mannosidosis type 2 and two causative mutations.

Patients and Methods: A 45-year old man (Patient A) and his 51-year old sister (Patient B) were investigated. Neurological, psychiatric, electrophysiological, biochemical and genetic tests were performed. MAN2B1 gene was analysed by Sanger sequencing. Segregation analysis was completed in the mother.

Results: Patient A had macrocephaly, coarse face, hypacusis and hepatomegaly in early childhood. Gait and limb ataxia, mild cognitive impairment occured in adulthood. Patient B has prominent forehead, hypacusis, generalized muscular atrophy and lower limb paresis in early childhood. Delusions with a diagnosis of schizophrenia and multiple joint deformities appeared at young adulthood. Brain MRI detected cerebellar atrophy, X-ray showed multiple sclerotic-edged cysts in humerus. Significant loss of axon of motoneurons was found in lower limbs according to the ENG.The alpha-mannosidase activities were decreased, under 2% of the health control. The serum and urine oligosaccharide analysis showed abnormal patterns. The siblings are compound heterozygous for c.283G>C(Ala95Pro) and c.523G>A(Gly175Arg), which are likely pathogenic variants according to the scientific databases. The mother is heterozygous for c.283G>C(Ala95Pro).

Conclusions: Based on family and clinical history, type 2 alpha mannosidosis was confirmed in the siblings with novel compound heterozygous mutations classified as pathogenic. The result of the segregation analysis strengthened the mutations in trans.This study was supported by KTIA_13_NAP-A-III/6; KTIA_NAP and with FIKP program.

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P11.011.A Preclinical studies on Alport Syndrome mice treated with chemical chaperons

Pavlos Ioannou[1], *Christoforos Odiatis*[1], *Apostolos Malatras*[1], *Kyriaki Antoniadou*[1], *Myrtani Pieri*[2], *Gregory Papagregoriou*[1], *Kostas Stylianou*[3], *Costantinos Deltas*[1]

[1]*University of Cyprus, Nicosia, Cyprus,* [2]*University of Nicosia, Nicosia, Cyprus,* [3]*University of Crete Medical School, Heraklion, Greece.*

Introduction: Alport Syndrome (AS) is a severe inherited glomerulopathy caused by mutations in the genes encoding the α-chains of type IV collagen, the most abundant component of the glomerular basement membrane (GBM). Alport patients lack effective therapies beyond blockade of the renin-angiotensin system.

Materials and Methods: This work describes the repurposing of two FDA-approved chemical chaperones (4-PBA and TUDCA) to the rescue of two AS mouse models: one that carries the Col4a3-p.Gly1332Glu in homozygosity and one that carries the Col4a3-p.Gly1332Glu substitution in compound heterozygosity with a Col4a3 knocked-out allele. Mice from each group were treated with chaperones or vehicle for 2, 6 or 12 months.

Results: Electron microscopy studies showed that the GBM of the 4-PBA treated AS mice after the 6-12-month treatment has a considerable improvement in the morphology, compared with placebo-treated or TUDCA-treated mice AS mice. Importantly, EM measurements displayed a 43% reduction of lesions and a significant decline of the lesions-severity in the GBM of 4-PBA treated mice. No adverse effects were noted in the GBM of the chaperone-treated wild type mice. Additionally, albuminuria and serum urea after a 12-month treatment of mice with 4-PBA have not reached the high values demonstrated by the non-treated AS mice (p-value <0.01).

Conclusions: The 6-12-month treatment with 4-PBA could effectively restore to a sufficient degree the morphology of GBM in both AS mouse models. Grants: Funded by the Alport Syndrome Foundation, Inc. (ASF), Pedersen Family and the Kidney Foundation of Canada (KFOC) and by the Cyprus Research and Innovation Foundation.

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P11.013.C Detection of giant chromosomal material on 7p+ with conventional karyotyping and aCGH

G. Ilieva, *E. Sukarova-Angelovska, D. Nestoroska, Lj. Muaremoska-Kanzoska, M. Pesevska, V. Anastasovska*

Genetic laboratory, University Clinic for Pediatrics, Ss. Cyril and Methodius University in Skopje, Faculty of Medicine, Skopje, Macedonia, The Former Yugoslav Republic of.

Introduction: Array CGH is widely used in cytogenetics centers for postnatal constitutional genome analysis with higher resolution than conventional karyotyping in patients with intellectual disabilities and multiple malformations. The technique represents an unsurpassed tool for detecting copy number variations (CNVs) and reveal origin of derivative chromosomes.

Materials and Methods: aCGH analysis was performed on a DNA sample from a 5-year-old child using the Affymetrix® CytoScan™ 750K Array (Applied Biosystems). Each array was consisted of 200k SNP and 550k non-polymorphic markers. The data was analyzed and interpreted using Chromosome Analysis Suite (ChAS) Software (v4.0).

Results: The clinical manifestation of the patient included major developmental delay, hypotonia, inability to sit and walk independently, absence of speech and facial dysmorphia. Karyotype showed mosaic presentation of 46,XY,der(7)(p+) in 10% of the cells. Since the mother had normal karyotype and father was unavailable for eventual translocation, array CGH was performed with the presence of major duplication of 7p21.3p11.2 encompassing 46,925 kbp, with 334 genes, estimated as 90% of the cells. Duplication of 7p is rarely described in the literature, with variable phenotypic spectrum depending on mosaicism and duplicated region.

Conclusion: Microarray-based comparative Genomic Hybridization (aCGH) is essential for evaluating derivative chromosomes of unknown origin. The reason for different level of mosaicism of partial trisomy 7p in our case with both techniques remains unexplained. One of the explanations is that cells with derivative chromosomes divide rarely in cell cultures, leading to conclusion that aCGH is more accurate technique for detecting mosaic chromosomal imbalances.

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P11.014.D Clinical and genomic characterization of 7q31.1 microduplication in a patient with developmental and neurological disabilities

Dragica Nestoroska[1], *V. Anastasovska*[1], *E. Sukarova-Angelovska*[1], *M. Pesevska*[1], *A. Veseli*[2], *G. Ilieva*[1]

[1]Genetic Laboratory, University Clinic for Pediatrics, Ss. Cyril and Methodius University in Skopje, Faculty of Medicine, Skopje, Macedonia, The Former Yugoslav Republic of, [2]University Institute of Clinical Biochemistry, Ss. Cyril and Methodius University in Skopje, Faculty of Medicine, Skopje, Macedonia, The Former Yugoslav Republic of.

Introduction: Array Comparative Genomic Hybridization (aCGH) represents molecular cytogenetic approach for genome-wide detection of copy number variants (CNVs) that allows efficient genetic diagnosis of pathological conditions, such as developmental delay and neurological disabilities. This technique is highly recommended as a first-tier diagnostic test for clinically relevant CNVs due to its cost and time efficiency, as well its high detection rate compared to conventional karyotyping.

Materials and Methods: The aCGH technique was used to determine the genetic background of dysmorphic, developmental and neurological abnormalities in a 5-year-old female Macedonian patient. The blood-derived DNA sample was analyzed using Affymetrix® CytoScanTM 750K Array (Applied Biosystems) that includes 550 k non-polymorphic and 200 k SNP markers. The data were interpreted using Chromosome Analysis Suite (ChAS) Software (v4.0).

Results: The patient showed the following clinical conditions: developmental delay, epileptic seizures, frequent convulsions, as well several dysmorhological features (macrocephaly, dolichocephaly, antimongoloid slanted eyes and micrognathia).The karyotyping demonstrated 47,XXX result, which did not correspond to the phenotype. The aCGH analysis revealed additional CNV represented as pathological microduplication occurring at the 7q31.1 cytoregion (513 kb, including *IMMP2L* and *LRRN3* genes). According to OMIM and ClinVar database for pathological gene expression, microduplication of the *IMMP2L* gene could be responsible for the severe neurodevelopmental disorders of the patient.

Conclusion: The aCGH technique is a high-resolution laboratory setting that allows detection of pathological CNVs. Further studies are needed for complete understanding of the mechanism related to gene duplication in the onset and progression of the presented developmental and neurological disorders.

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P11.016.B Further delineation of the clinical spectrum of variants in the *ASCL1* gene

Margot Grisval[1], *Emma Walkeling*[2], *Odile Boespflug-Tanguy*[3], *Ha Thi-Tuyet Trang*[4], *Aurore Garde*[1], *Christophe Philippe*[5], *Adélaide Rega*[6], *Marie Gabrielle Mourot De Rougemont*[6], *Véronique Darmency*[7], *Candace Ben Signor*[8], *Anne Houzel*[9], *Frédéric Huet*[10], *Yannis Duffourd*[5], *Christel Thauvin-Robinet*[1,11], *Tashunka Taylor-Miller*[12], *Louise Busby*[12], *Laurence Faivre*[1,11]

[1]Centre de Référence Anomalies du Développement et Syndromes Malformatifs, FHU TRANSLAD, Hôpital d'Enfants, CHU Dijon, Dijon, France, [2]Consultant in Clinical Genetics, North East Thames Regional Genetics Service, Great Ormond Street Hospital for Children NHS Foundation Trust, Great Ormond Street, London, United Kingdom, [3]Centre de référence des leucodystrophies, service de Neurologie Pédiatrique et Maladies métaboliques, Hôpital Robert Debré, AP-HP, Paris, France, Paris, France, [4]Service de Physiologie – Explorations Fonctionnelles, Hôpital Robert Debré, AP-HP, Paris, France, Paris, France, [5]Inserm UMR1231 GAD et UF6254, CHU de Dijon et Université de Bourgogne, Dijon, France, Dijon, France, [6]Service de Radiologie Pédiatrique, Hôpital d'Enfants, CHU Dijon, Dijon, France, Dijon, France, [7]Service de Neurophysiologie Clinique Adultes et Enfants, Hôpital d'Enfants, CHU Dijon, Dijon, France, Dijon, France, [8]Centre de Compétences Maladies Endocriniennes De La Croissance et du Développement, Hôpital d'Enfants, CHU Dijon, Dijon, France, Dijon, France, [9]Pneumologie Pédiatrique, Hôpital d'Enfants, CHU Dijon, Dijon, France, Dijon, France, [10]Pédiatrie pluridisciplinaire, Hôpital d'Enfants, CHU Dijon, Dijon, France, Dijon, France, [11]Inserm UMR1231 GAD, Génétique des Anomalies du Développement, Université de Bourgogne, Dijon, France, Dijon, France, [12]Rare & Inherited Disease Laboratory, London North Genomic Laboratory Hub, Great Ormond Street Hospital for Children NHS Foundation Trust, London WC1N 3BH, UK, London, United Kingdom.

Introduction: The ASCL1-HOX2A-PHOX2B developmental cascade has been proposed as a candidate pathway for Congenital central hypoventilation syndrome (CCHS) and Haddad syndrome (CCHS associated to Hirschprung disease). *ASCL1* has been described in association with both CCHS and Haddad syndrome. However, only 3 patients have been described in the literature to date (OMIM# 100790).

Patients and methods: A French and a UK patient were referred to their genetic centres for investigation of syndromic Hirschsprung disease. Trio whole exome or genome sequencing was performed and details of the *ASCL1* variants submitted to the GeneMatcher data sharing platform in both cases.

Results: The patients were two boys aged 9 and 6 years old respectively. Their common phenotype includes Hirschprung disease, strabismus, cardiac dysautonomy and learning disability with speech delay. Neither patient has CCHS. The older patient is under investigation for sleep apnea and has stable T2 hyperintensity in the dentate nuclei on brain MRI. The younger patient has a normal sleep study and brain MRI scan. Both patients have the same *de novo* heterozygous missense *ASCL1* variant (NM_004316.3:c.379G> A p.(Glu127Lys)). This variant is absent from the population database GnomAD and affects a conserved amino acid located in the functional domain HLH. Multiple *in silico* tools predict pathogenicity.

Conclusion: We report two patients with the same *de novo* heterozygous *ASCL1* variant and a strikingly similar clinical phenotype. This observation suggests that the phenotypic spectrum associated with *ASCL1* gene variants is broader than previously reported. Additional patients, as well as functional studies will be required.

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P11.017.C Baraitser-Winter cerebrofrontofacial syndrome the first proved case in Bulgaria

Tsvetina Ivanova Veleva, *Hadil Kathom, Trayan Delchev, Daniela Avdjieva-Tzavella*

SBALDB, Medical university - Sofia, Sofia, Bulgaria.

Introduction: Baraitser-Winter cerebrofrontofacial syndrome (BWCFFS) is a rare multisystem developmental disorder characterized by distinctive craniofacial features, intellectual disability, ocular colobomata, hearing loss, short stature, brain malformation, epilepsy, structural anomalies of palate, heart and kidneys. BWCFFS is caused by mutations in two different genes - ACTB and ACTG1, that encode β- and γ-actins. Individuals with ACTB mutations seem to have more severe phenotype but ACTG1

pathogenic variants appear to have more frequent CNS involvement.

Materials and Methods: We present a 13-year-old boy with typical craniofacial characteristics (coarse facial features, highly arched eyebrows, widely spaced eyes, prominent nasal bridge, wide mouth, retrognatia, dysplastic ears, skeletal abnormalities) and mild intellectual disability. The patient had a normal male karyotype. Results from multiplex ligation-dependent probe amplification analysis, glucosaminoglycans in urine, echocardiography, renal ultrasound scanning, X-ray examination of the spine and cranium were normal.

Results: The molecular genetic analysis was performed by NGS (Baraitser-Winter Cerebrofrontofacial Syndrome Panel). This diagnostic test evaluated 3 genes - ACTB, ACTG1 and ANKRD11. The data interpretation revealed a likely pathogenic variant - c.220G>A (p.Gly74Ser) in ACTB gene.

Conclusions: Nearly all reported cases of BWFFS syndrome occur as a result of de novo mutations. The identified variant is not present in population databases. Our study further expanded the genotypic spectrum and genotype-phenotype correlations of BWCFFS.

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P11.018.D Clinical and molecular characterization of Beckwith-Wiedemann spectrum patients conceived through assisted reproductive technology

Diana Carli[1], *Jennifer Spina*[1], *Silvia Russo*[2], *Guido Cocchi*[3], *Donatella Milani*[4], *Elisabetta Prada*[4], *Daniela Melis*[5], *Luigi Tarani*[6], *Marina Macchiaiolo*[7], *Angela Sparago*[8], *Laura Pignata*[8], *Pierpaola Tannorella*[2], *Simona Cardaropoli*[1], *Andrea Bartuli*[7], *Andrea Riccio*[8], *Alessandro Mussa*[1], *Giovanni B. Ferrero*[1]

[1]*University of Torino, Torino, Italy,* [2]*Istituto Auxologico Italiano, Milano, Italy,* [3]*University of Bologna, Bologna, Italy,* [4]*Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy,* [5]*Federico II University, Napoli, Italy,* [6]*"Sapienza" University of Rome, Roma, Italy,* [7]*Bambino Gesù Children's Hospital, Roma, Italy,* [8]*Università degli Studi della Campania "Luigi Vanvitelli", Caserta, Italy.*

Introduction: Beckwith-Wiedemann spectrum (BWSp) prevalence is tenfold increased in children conceived through assisted reproductive techniques (ART). More than 90% of ART-BWSp patients reported so far carry Imprinting Center 2 Loss-of-Methylations (IC2-LoM), versus ≃50% of naturally conceived BWSp patients.

Methods: clinical and molecular features of 50 ART-BWSp patients were reviewed.

Results: Thirty-five patients (70%) carried IC2-LoM, 11 (22%) chromosome 11 paternal uniparental disomy (UPD(11)pat), 1 (2%) Imprinting Center 1 Gain-of-Methylation (IC1-GoM); 2 (4%) tested negative and 1 (2%) refused testing (the latter were clinically diagnosed with BWSp score ≥ 4). Macroglossia was observed in 39 patients (78%), lateralized overgrowth in 30 (60%), omphalocele in 9 (18%) and prolonged hyperinsulinism in 1 (2%). Malignancies (1 Wilms tumor and 1 hepatoblastoma) occured in 2 patients (4%) carrying UPD(11)pat. Data on the type of ART were available in 22 patients: 13 were born from homologous in vitro fertilization (IVF), 4 from homologous intracytoplasmic sperm injection (ICSI), 4 from ICSI with egg donation, and 1 from intra-uterine insemination (IUI). Infertility cause was unknown in 13/22 cases (59.1%), maternal in 4/22 cases (18.2%; 3 bilateral salpingectomies and 1 endometriosis) and paternal in 5/22 cases (22.7%; all presenting oligo/azoospermia).

Conclusions: We found a 22% of UPD(11)pat in our cohort, a fraction consistent with naturally conceived BWSp and strongly contradicting literature, reporting almost invariably only IC2-LoM cases in ART-BWSp cohorts. Since UPD(11)pat likely results from postfertilization recombination, this finding allows to hypothesize that complex molecular mechanisms, besides methylation disturbances, may underlie BWSp increased risk in ART pregnancies.

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P11.019.A 11p15 imprinting defects and phenotype expression in 12 patients with Beckwith-Wiedemann syndrome

Ivona Sansovic, *Ljubica Boban, Mijana Kero, Ingeborg Barisic*

Children's Hospital Zagreb, Scientific Centre of Excellence for Reproductive and Regenerative Medicine (CERRM), University of Zagreb School of Medicine, Zagreb, Croatia.

Introduction: Beckwith-Wiedemann syndrome (BWS) is caused by genetic or epigenetic changes that modify expression of genes in the imprinted region of chromosome 11p15.5. Although recent studies proved the association between 11p15.5 region defect and BWS clinical features the complex genotype-phenotype relationship is still challenging.

Materials and method: The phenotypes of 12 BWS patients with molecular defect in imprinted 11p15.5 region, detected by Methylation-Specific Multiplex Ligation-dependent Probe Amplification method, were compared with the literature reports on genotype-phenotype correlations in four molecular subclasses: loss of methylation at imprinting center 2 (IC2-LoM, n = 8), gain of methylation at imprinting center 1 (IC1-GoM, n = 1), chromosome 11p15 paternal uniparental disomy (UPD, n = 2) and duplication of paternal IC1 (IC1-dup, n = 1).

Results: Macrosomia, macroglossia and neonatal hypoglycemia were dominant findings across all subclasses. Hemihyperplasia was present in both UPD patients. Renal and urinary anomalies, umbilical hernia, organomegaly and increased cancer risk typical for IC1-GoM cases were observed in IC1-GoM patient. Omphalocele, ear anomalies, nevus flammeus, preterm birth, and the use of assisted reproductive technologies were more common in IC2-LoM genotype. IC1-dup patient have a few BWS characteristic features.

Conclusion: Our findings are in accordance with the largest recent correlation studies on BWS and support the hypothesis that different 11p15 alterations are associated with specific phenotypes in BWS. Acknowledgment: This work was supported by Scientific Center of Excellence for Reproductive and Regenerative Medicine and by the EU through the European Regional Development Fund, under grant agreement No. KK.01.1.1.01.0008, project, Reproductive and Regenerative Medicine - Exploring New Platforms and Potentials".

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P11.020.B Phenotypically concordant but epigenetically discordant monozygotic dichorionic diamniotic twins with Beckwith-Wiedemann syndrome

Hidenobu Soejima[1], *Feifei Sun*[1,2], *Hitomi Yatsuki*[1], *Ken Higashimoto*[1], *Satoshi Hara*[1]

[1]Saga University, Saga, Japan, [2]Shengjing Hospital of China Medical University, Shenyang, China.

Beckwith-Wiedemann syndrome (BWS) is an imprinting disorder with complex and diverse phenotypes caused by (epi)genetic alterations. The incidence of monozygotic (MZ) twins in BWS patients is higher than in the general population. Most MZ twins with BWS are female and have phenotypical discordance: one of the twins is clinically diagnosed with BWS, while the other shows a mild phenotype or a completely normal phenotype. The most frequent (epi)genetic alteration in MZ twins is loss of methylation of imprinting control region 2 (ICR2-LOM) at 11p15.5. Intriguingly, ICR2-LOM is usually found in the peripheral blood leukocytes (PBL) of both twins, even if they are clinically discordant. Here, we present a rare pair of MZ dichorionic diamniotic female twins with BWS and concordant phenotypes (a Beckwith-Wiedemann spectrum (BWSp) score of 5 in each twin). Molecular analysis of genomic DNA from PBL revealed ICR2-LOM in one twin but not the other. Our analyses suggest that ICR2-LOM occurred between days 1 and 3 after fertilization, followed by twinning and an even mosaic distribution of ICR2-LOM cells at the tissue level between the twins, except in hematopoietic stem cells, during embryogenesis.

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P11.021.C Refinement of the 22q11 duplicated phenocritical locus in bladder exstrophy epispadias complex

Glenda Maria Beaman[1,2], *William G. Newman*[1,2], *Woolf S. Adrian*[1,2], *Raimondo M. Cervellione*[2], *David Keene*[2], *Imran Mushtaq*[3]

[1]University of Manchester, Manchester, United Kingdom, [2]Manchester University NHS Foundation Trust, Manchester, United Kingdom, [3]Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom.

The bladder exstrophy-epispadias complex (BEEC) comprises of a spectrum of anterior midline defects affecting the bladder or urethra. Despite its clinical importance, the causes of bladder exstrophy remain undefined. BEEC affects 1 in 10,000 births, with a twofold higher incidence in males. Duplications of chromosome 22q11.2 have been identified in approximately 3% of BEEC cases. This is the strongest reported association with BEEC, conferring a greater than twelvefold risk. A comparison of eight previously reported 22q11.21 duplications in individuals with CBE defined a 414 kb 'phenocritical' region, harboring 10 candidate protein coding genes. By undertaking CNV analysis in BEEC patients using the Infinium CytoSNP-850K v1.2 Beadchip Kit (Illumina), we identified a British female with classic bladder exstrophy (CBE) with a maternally inherited 313kb duplication, which refines the phenocritical region, to include only five of the protein coding genes previously identified: *CRKL, AIFM3, LZTR1, THAP7* and *P2RX6*. The duplication lies within a single regulatory chromosomal topographical active domain. Subsequent copy number analysis using TaqMan assays for these five genes in 116 individuals with BEEC revealed no evidence of single gene duplications. RNA array data from embryonic mice in GenitoUrinary Development Molecular Anatomy Project (GUDMAP) detected *CRKL*, *LZTR1* and *THAP7* expressed in embryonic kidney mesenchyme and interstitium and tubules, embryonic ureter and embryonic bladder. These data refine the size of the duplication at the 22q11.21 also suggesting that *CRKL*, *THAP7*, and *LZTR*1 are CBE candidate genes and contribute to the potential disease-associated mechanism predisposing to BEEC at this locus.

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P11.022.D Understanding the new *BRD4*-related Cornelia de Lange-like syndrome: clinical, genomic and bioinformatic delineation with an international cohort study

Guillaume Jouret[1], *Solveig Heide*[2], *Arthur Sorlin*[1,3,4], *Laurence Faivre*[3,4], *Sandrine Chantot-Bastaraud*[5], *Marie Denis-Musquer*[6], *Peter D. Turnpenny*[7], *Charles Coutton*[8], *Gaëlle Vieville*[8], *Julien Thevenon*[8], *Austin Larson*[9], *Florence Petit*[10], *Elise Boudry*[11], *Thomas Smol*[11], *Bruno Delobel*[12], *Bénédicte Duban-Bedu*[12], *Chiara Fallerini*[13], *Francesca Mari*[13], *Caterina Lo Rizzo*[14], *Alessandra Renieri*[13,14], *Jean-Hubert Caberg*[15], *Frederic Tran Mau-Them*[4,16], *Isabelle Maystadt*[17], *Philippe Theis*[1], *Christian Müller*[1], *Didier Menzies*[18], *Dominique Bourgeois*[1], *Emmanuel Scalais*[19], *Barbara Klink*[1]

[1]National Center of Genetics (NCG), Laboratoire national de santé (LNS), Dudelange, Luxembourg, [2]Service de Génétique Cytogénétique Embryologie Hôpital Pitié-Salpétrière, Paris, France, [3]Centre de Génétique, CHU de Dijon, Dijon, France, [4]Génétique des Anomalies du Développement, Inserm 1231 GAD, Université de Bourgogne, Dijon, France, [5]Service de génétique et embryologie médicales, CHU Paris Est, Hôpital d'Enfants Armand-Trousseau, Paris, France, [6]Service d'Anatomie et Cytologie Pathologiques, Hôpital-Dieu, Nantes, France, [7]Clinical Genetics Department, Royal Devon and Exeter Hospital, Exeter, United Kingdom, [8]Service de Génétique Médicale, Grenoble, France, [9]Clinical Genetics Department, Children's Hospital Colorado, Littleton, CO, USA, [10]Clinique de Génétique « Guy Fontaine », CHU de Lille, Lille, France, [11]Institut de Génétique Médicale, CHU de Lille, Lille, France, [12]Centre de Génétique Chromosomique, GH de l'Institut Catholique de Lille, Lille, France, [13]Medical Genetics Department, University of Siena, Siena, Italy, [14]Genetica Medica, Azienda Ospedaliera Universitaria Senese, Siena, Italy, [15]Centre de Génétique Humaine, CHU de Liège, Liège, Belgium, [16]Unité Fonctionnelle 6254 d'Innovation en Diagnostique Génomique des Maladies Rares, Pôle de Biologie, CHU Dijon Bourgogne, Dijon, France, [17]Centre de Génétique Humaine, Institut de Pathologie et de Génétique (IPG), Gosselies, Belgium, [18]National Center of Pathology (NCP), Laboratoire national de santé (LNS), Dudelange, Luxembourg, [19]Pediatric Neurology Unit, Pediatric Department, Centre Hospitalier de Luxembourg, Luxembourg, Luxembourg.

Introduction: To date, only three patients have been reported in the literature with *BRD4* point mutations. However, a small but growing body of scientific literature is emerging about clinical findings in patients with 19p13.12 deletions overlapping *BRD4*, of which nine patients have been described. They share a characteristic common phenotype including growth retardation, microcephaly, intellectual disability, cardiac defects and facial dysmorphism suggestive of Cornelia de Lange syndrome (CdLS). Interestigly, CdLS is often caused by mutations in *NIPBL*, which has recently been shown to interact closely with *BRD4*.

Methods: To characterize this novel syndrome, we formed an international collaborative study, and collected twelve new patients: six with 19p13.12 deletions overlapping *BRD4* and six with *BRD4* point mutations. We performed phenotype and genotype analysis, critical region delineation and assessment of the impact of structural variants on three-dimensional chromatin interactions by Topologically Associating Domains (TADs) analysis. We assessed the participation of contiguous genes never associated with human diseases before, by using the data-mining software Manteia to compare with phenotypes observed in murine knockout models.

Results: We report the first cohort of patients with *BRD4*-related Cornelia de Lange-like syndrome and describe new cardinal clinical findings. By integrating prenatal findings from fetopathological examinations, phenotypes of pediatric patients and adults,

we describe the specific evolution of dysmorphic features during the different stages of life.

Conclusion: *BRD4*-related phenotype is part of the CdLS spectrum but is characterized by clinically relevant specificities that distinguish it from other cohesinopathies.

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P11.023.A miRNA-free rare pathogenic CNVs could drive toward variable CAKUT phenotypes

Ivan Zivotic, *Ivana Kolic, Kristina Popic, Jelena Filipovic Trickovic, Ana Djordjevic, Maja Zivkovic, Aleksandra Stankovic, Ivan Jovanovic*

"Vinca" Institute of nuclear sciences, Insltitute of the national interest of the Republic of Serbia, University of Belgrade, Belgrade, Serbia.

Introduction: Genetic studies of congenital anomalies of the kidney and urinary tract (CAKUT) have demonstrated variable penetrability and expressivity of the associated genetic defects. Previously, it was shown that deletions of 17q12 and 22q11.2 regions were specific for kidney anomalies (KA) while 16p11.2 and 1q21.1 loci showed extensive pleiotropy in CAKUT phenotypes. CNVs affecting miRNA gene dosage have been described to have functional influence on gene expression. We aimed to conduct comprehensive in silico analysis using publicly available databases to analyze miRNA content of CAKUT-associated CNVs in quoted chromosomal loci with regard to pleiotropy.

Methods: Extensive literature review was conducted to collect data about pathogenic rCNVs associated with CAKUT. UCSC genome browser tool was employed for mapping miRNAs onto collected rCNV regions.

Results: Analysis of CNVs in CAKUT included four studies counting more than 2500 patients. In further analysis we included 191 patients harboring pathogenic CNVs. Surprisingly, CAKUT pleiotropic regions (16p11.2, 1q21.2) did not contain any miRNA. 22q11.2 showed the densest miRNAs content (n = 21).

Conclusions: Absence of miRNAs may potentially pronounce the pleiotropy of the CAKUT genetic defects, thus leading to the variety of phenotypes. Contrary, abundancy of miRNAs in 22q11.2 might be associated with reproducible phenotype, such as KA, producing the functional effect when deleted. This assumption agrees with recent results of miRNA expression variability in 22q11.2 deletion syndrome. Acknowledgements: This research was supported by the Science Fund of the Republic of Serbia, PROMIS, # 6066923, miFaDriCa, and Serbian Ministry of Education, Science and Technological development.

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P11.024.B Are miR-548 family members potential genetic drivers of CAKUT

Kristina Mitrovic, *Ivana Kolic, Ivan Zivotic, Jelena Filipovic Trickovic, Ana Djordjevic, Maja Zivkovic, Aleksandra Stankovic, Ivan Jovanovic*

Institute of Nuclear Sciences "Vinca", Belgrade, Serbia.

Introduction: miR-548 family members, located on all human chromosomes except chr19 and chrY, regulate podocyte differentiation in vitro, important for kidney development. Rare copy number variants (rCNVs) are the common genetic cause of Congenital Anomalies of the Kidney and Urinary Tract (CAKUT) and could harbour miRNAs. The aim of this study was to investigate to which extent rCNVs associated with CAKUT harbour miR-548 members.

Materials and Methods: Extensive literature review was conducted to collect data of pathogenic and likely pathogenic rCNVs in CAKUT patients. UCSC genome browser tool was employed for mapping of miR-548 members onto collected rCNV regions and gnomAD SV controls database. Bioinformatic analysis was conducted using miRPathDB2 tool.

Results: We generated CAKUT database of pathogenic CNVs in 79 chromosome regions from 191 patient and likely pathogenic CNVs in 74 regions from 87 patients. Pathogenic rCNVs of seventeen patients, located on 7 chromosomes, contained at least one miR-548 member. Likely pathogenic rCNVs of 4 patients, located on 3 chromosomes, contained one of miR-548 members. Bioinformatic analysis implied the role of mapped miRNAs in the regulation of processes associated with CAKUT. In controls, only hsa-mir-548i-3 (out of 73 precursors) was mapped on polymorphic CNVs (af>1%) and wasn't identified in patients.

Conclusions: miR-548 members located in rCNVs should be investigated in future studies as potential genetic drivers of CAKUT development, beyond protein coding genes. Acknowledgements: This research was supported by the Science Fund of the Republic of Serbia, PROMIS, #6066923, miFaDriCa, and Serbian Ministry of Education, Science and Technological development.

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P11.025.C Preeclampsia as a potential clinical feature in Cantú syndrome

Eirny Thorolfsdottir, *Svanborg Gisladottir, Hans T. Bjornsson*

Landspitali University Hospital, Reykjavik, Iceland.

Introduction: Cantú syndrome (CS) is caused by gain-of-function pathogenic disease-causing variants in the genes coding for ABCC9 and KCNJ8, which together form an ATP-sensitive potassium channel. CS is a rare systemic syndrome with a great clinical variability, characterized by coarse facies, hypertrichosis, osteochondrodysplasia and cardiac anomalies. We present a family with eight individuals with CS for which the proband was initially diagnosed with Beckwith-Wiedemann syndrome. Whole genome trio sequencing revealed a likely pathogenic missense variant in the *ABCC9* gene (NM_005353286.2); c.1745T>A (p.Val582Asp), which the boy shares with seven similarly affected family members (patent ductus arteriosus, pericardial effusion, cardiomegaly, coarse facial features and hypertrichosis). Premature births, polyhydramnios and large for gestational age newborns are perinatal factors also seen in the family. Furthermore, maternal preeclampsia (n = 4) or hypertension during pregnancy (n = 1) is observed in 5 of 6 cases (83%) in this family, when CS mothers carry CS fetuses.

Discussion: Over-activity of the K_{ATP} channel and dysregulation of renin-angiotensin signaling (RAS) triggers cardiac hypertrophy, and dysregulation of RAS is also one of multiple factors thought to contribute to the development of preeclampsia. Here we present

three CS mothers who experience preeclampsia when carrying affected CS fetuses.

Conclusion: Preeclampsia as well as maternal preeclampsia has previously been described for other syndromes, but to our knowledge preeclampsia has not been associated with CS before, and we speculate that maternal preeclampsia might be an expansion of the CS phenotypic spectrum. If true, studies of CS may yield insights into the mechanistic basis of preeclampsia.

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P11.026.D *ATP1A3* gene is responsible for isolated and syndromic auditory neuropathy (CAPOS syndrome)

Margaux Serey-Gaut[1], Sophie Achard[2], Laurence Jonard[3], Marine Parodi[2], Natalie Loundon[2], Christine Poncet[4], Bruno Eymard[5], Naima Deggouj[6], Yves Sznajer[7], ***Sandrine Marlin***[1]

[1]Hopital Necker, Service de Génétique Clinique, Paris, France, [2]Hopital Necker, Service d'ORL, Paris, France, [3]Hopital Necker, Service de Génétique Moléculaire, Paris, France, [4]Hopital Rothschild, Centre de Réglage des Implants Cochléaire, Paris, France, [5]Institut de Myologie, Unité clinique de pathologie neuromusculaire, Paris, France, [6]Cliniques Universitaires Saint Luc UCL, service d'ORL, Bruxelles, Belgium, [7]Cliniques Universitaires Saint Luc UCL, service de Génétique, Bruxelles, Belgium.

CAPOS syndrome combines progressive hearing loss (auditory neuropathy type, (AN)), optic atrophy, hypotonia, and cerebellar ataxia. The disorder is described as appearing in childhood, with acute episodes of febrile neurological deterioration resembling encephalitis.We conducted a cohort study of 39 families (43 patients) with isolated (73%) or syndromic (27%) AN without cochlear nerve malformation. Their DNA was analyzed by Next Generation Sequencing using a panel of 216 genes involved in isolated or syndromic deafness.Four unrelated patients had the same heterozygous pathogenic variant of the *ATP1A3* gene, c.2452G>A, p.(Glu818Lys), already reported as responsible for CAPOS syndrome (OMIM-601338). The diagnosis of the hearing loss was made in post-lingual period from 5 to 12 years old. The deafness progressively worsened with very low word recognition (10%) despite a classical hearing aid. A single or bilateral cochlear implantation allowed recovering a word recognition score close to 100% (up to 12 years post-implant). Two patients have never had any of the febrile episodes classically described. Optic nerve damage was not present in two patients, one of whom was 16 years old. The ataxia described in the CAPOS syndrome is attributed to cerebellar damage but the implication of a vestibular deficit was present in 2/3 of the patients tested.We have identified the *ATP1A3* p.(Glu818Lys) variant in patients with isolated neuropathy with or without inaugural febrile episodes. Balance disorders could involve peripheral vestibular damage. Cohort studies should confirm efficacy in auditory perception in these patients.

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P11.027.A A new ocular phenotype in cardiofaciocutaneous syndrome

Maria Tessitore[1], *Giulia Abbinante[1], Mariateresa Falco[2], Flavio Gallo[1], Carnem Plaitano[3], Dario Di Salvio[4], Adriano Magli[1], Daniela Melis[1]*

[1]Scuola Medica Salernitana, University of Salerno, Salerno, Italy, [2]Pediatric Unit San Giovanni di Dio e Ruggi d'Aragona University Hospital, Salerno, Italy, [3]3. Ophtalmology Department, San Giovanni di Dio e Ruggi d'Aragona University Hospital, Salerno, Italy, [4]4. Department of Maternal-Infant, Pediatric Section, University of Naples Federico II, Naples, Italy.

Introduction: Cardio-facio-cutaneous syndrome (CFC syndrome) is a congenital disorder belonging to RASopathies, a group of syndromes caused by mutations in the Ras/mitogen-activated protein kinase pathway. BRAF, MEK1 and MEK2 are the most frequently genes involved. The major clinical manifestations include craniofacial dysmorphic features, growth retardation with short stature, congenital heart diseases, neurodevelopment delay, hypotonia and dermatologic abnormalities. Ocular involvement occurs in the majority of individuals and include: strabismus, refractive errors, nystagmus, ptosis, and optic nerve hypoplasia. Case report. We describe a 5-year-old girl with a heterozygous pathogenic variant [c. 1741A>Gp. (Asn581Asp)] in BRAF gene.The patient presents typical dysmorphic features (thin and curly hair, bilateral epicanthum, macrostomia, macroglossia), congenital heart defects (atrial septal defect, pulmonary valve stenosis), and moderate developmental delay. MRI brain detected thinning of the corpus callosum and enlarged periventricular spaces. She also presents dental caries, follicular keratosis and laterocervical infantile hemangioma. Eye examination showed exotropia, anomalies of the visual evoked potentials and, in particular, excavation of optic disc. Excavation of optic disc is an ocular manifestation that has not been yet described in patients with CFC syndrome. This finding could be associated with optic nerve atrophy, previously found in some cases of CFC syndrome.

Conclusions: This case report suggest to carry out an accurate funduscopic examination in order to highlight alterations of the optic disc in patients with RASopathies to obtain a precocious diagnosis of optic nerve involvement.

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P11.028.B Identification of Cenani-Lenz syndrome due to compound heterozygous variant in APC

Jair A. Tenorio-Castaño[1,2], Pedro Arias[1,2], Julian Nevado[1,2], Natalia Gallego[1], Cristina Silvan[1], Sergio Ramos[1], Sixto García-Miñaúr[1,2], Nuria Rodriguez-Salas[3], Leopoldo Martínez[4], ***Pablo Lapunzina***[1,2]

[1]Medical and Molecular Genetics Institute (INGEMM), Madrid, Spain, [2]Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Madrid, Spain, [3]Hereditary cancer unit, Oncology, Hospital Universitario la Paz, Universidad Autónoma de Madrid (UAM), 28046., Madrid, Spain, [4]Department of Pediatric Surgery, Hospital Universitario La Paz, Madrid, Spain.

Cenani-Lenz (CLS) is an infrequent congenital malformation characterized by syndactyly of the hands with abnormalities of the forearm bones that can be also present in the lower limbs, renal abnormalities and dysmorphism. It is usually caused by autosomal recessive variants in *LRP4*, and duplication encompassing *GREM1* and *FMN1*. More recently, *APC* pathogenic variants, mostly truncating, have been also suggested to be related with CLS. APC acts on the WNT signaling pathway as well as LRP4, which seems to be the canonical pathway associated with CLS. Here, we report a family in which the index case was diagnosed with multiple adenomatous polyposis, syndactyly, xerosis cutis

and ADHD. Whole exome sequencing revealed that the proband have two variants in the *APC* gene, one nonsense (NM_001354906.2:c.1564C>T: p.Arg522*) at exon 17 and one intronic variant NM_001354906.2:c.-191T>A. One variant was de novo and the second one inherited from the mother. None of the variants were detected in the pseudocontrol population databases (gnomAD exomes, gnomAD genomes, 1000G, ESP, Kaviar) and the majority of the pathogenic predictors suggested a pathogenic effect for the nonsense variant. In silico analysis of the intronic variant suggested that removed the ATG, thus having a damaging effect at the ORF, based on the 5'UTR location of the variant. In this region, other variants have been previously reported as pathogenic. Altogether suggest that for the first time, a compound heterozygous variant in APC is described in a case with Cenani-Lenz, expanding the phenotype and molecular features associated with the disease.Grants: FIS-PI20/01053

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P11.030.D Vascular, skeletal and endocrine anomalies in mosaic variegated aneuploidy syndrome 2 caused by biallelic variants in *CEP57*

María Palomares-Bralo[1,2,3], *Marta Pacio-Míguez*[1], *Anna María Cueto-González*[4,5,6], *Sixto García-Miñaúr*[1,2,3], *Ángela del Pozo*[1,2], *Juan José Menéndez Suso*[7], *Francisco J. Climent Alcalá*[8], *Elena Mansilla*[1,2,3], *Cristina Schuffelmann*[7], *María Dolores Lledín*[9], *Mario Solís*[1,2], *Teresa López*[1], *Patricia Valcorba*[1,2,3], *Sofía Siccha*[10], *María Irene Valenzuela Palafoll*[4,11], *Fermina López Grondona*[4], *Francisca Nieto Aranda*[1,11], *Eduardo Tizzano*[4,11], *Pablo Lapunzina*[1,2,3], *Fernando Santos-Simarro*[1,2,3]

[1]*Instituto de Genética Médica y Molecular (INGEMM) Hospital Universitario La Paz, Madrid, Spain,* [2]*CIBERER, Centro de Investigación Biomédica en Red de Enfermedades Raras, ISCIII, Madrid, Spain,* [3]*European Reference Network Congenital Malformations & Rare Mental Disability (ERN -ITHACA), Madrid, Spain,* [4]*Departamento de Genética Clínica y Molecular, Campus del Hospital Vall d'Hebron, Barcelona, Spain,* [5]*Grupo de Genética Médica, Instituto de Investigación Vall Hebron (VHIR), Campus del Hospital Vall d'Hebron, Barcelona, Spain,* [6]*European Reference Network Craniofacial Anomalies and ENT disorders (ERN-CRANIO), Barcelona, Spain,* [7]*Servicio de Cuidados Intensivos Pediátricos, Hospital Universitario La Paz, Madrid, Spain,* [8]*Unidad de patología compleja pediátrica, Hospital Universitario La Paz, Madrid, Spain,* [9]*Unidad de hepatología pediátrica, Hospital Universitario La Paz, Madrid, Spain,* [10]*Servicio de pediatría, Hospital Universitario La Paz, Madrid, Spain,* [11]*Grupo de Genética Médica, Instituto de Investigación Vall Hebron (VHIR), Barcelona, Spain.*

Introduction: Mosaic variegated aneuploidy (MVA) syndrome is a rare autosomal recessive disorder characterized by a variable percentage of chromosome gains and losses in somatic cells, leading to constitutional mosaic aneuploidies. Biallelic mutations in *BUB1B*, *CEP57* and *TRIP13* have been identified as the underlying cause of MVA 1 (OMIM 257300), MVA 2 (OMIM 614114) and MVA 3 (OMIM 617598) respectively. Patients with MVA present with a non-distinctive phenotype with pre- and postnatal growth retardation, microcephaly, intellectual disability, skeletal anomalies, facial dysmorphisms, heart defects, seizures, hypothyroidism, ocular defects and childhood cancers associated to *BUB1B* and *TRIP13*. Biallelic *CEP57* variants have only been described in 10 patients so far, and the phenotype is poorly known.

Materials and Methods: We have performed a retrospective analysis on the phenotype of all reported cases presenting with MVA2 and pathogenic variants in *CEP57* and describe the clinical findings of 2 additional unrelated patients with MVA2 identified through WES.

Conclusions: Analysis of these 12 cases delineates a complex phenotype that includes pre and postnatal growth retardation and characteristic facial features. Cardiac, vascular and skeletal malformations also seem to be part of the phenotype, as well as endocrine abnormalities such as hypothyroidism and growth hormone deficit with or without pituitary anomalies. The identification of these manifestations will improve the clinical management of these patients. Grants: Raregenomics network, financed by the Consejería de Educación de la C. de Madrid (S2017/BMD-3721), ISC III, Ministerio de Ciencia e Innovación (PI19/01681) and the European Social Fund.

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P11.031.A Familial cervical ribs associated with azygos lobe

Nouha Bouayed ABDELMOULA, ***Amir Karra****, Balkiss ABdelmoula*

Genomics of signalopathies at the service of medicine UR17ES36, Medical University of Sfax, Sfax, Tunisia.

Objective: Cervical rib is a congenital over-development of the costal process of the 7th cervical vertebra (1 to 2% of the population) which is known to cause brachial plexopathy in up to 10% of the affected individuals. Cervical ribs may cause thoracic outlet syndromes by compression. Their origin is not well elucidated but it seems that genetic (HOX genes) and environmental factors (maternal exposure to chemicals or stress) may be involved. Familial cases with a suspected autosomal dominant inheritance were reported. Here, we describe a Tunisian pedigree in which a young female was diagnosed as having familial bilateral cervical ribs. This congenital abnormality was associated with another congenital variation of the lung (0.2-1.2% of the population) which was an azygos lobe of the right lung.

Material and Methods: A Tunisian 29-years-old female was referred to us for brachial plexopathy. Thoracic X-ray as well as cervical spine thoracic MRI and scan were performed.

Results: The interpretation of the chest X-ray revealed the presence of bilateral cervical ribs as well as a pulmonary opacity of the right apex. The MRI confirmed the presence of two supernumerary cervical ribs at the 7th cervical vertebra. There was also a Tornwaldt cyst which is a common incidental benign midline nasopharyngeal mucosal cyst. More over, there was a pulmonary nodule at the apical segment of the right lung. The thoracic scan identified the pulmonary lesion as an azygos lobe.

Conclusion: Our patient had cervical ribs inherited from her father associated to multiple other congenital conditions.

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P11.032.B Diagnostic WES-based gene panel testing in (non)-syndromic patients with cleft lip and/or palate in the Netherlands

*Inge L. Cox[1], **Lisca F. Fockema Wurfbain**[1], M F. van Dooren[2], V Verhoeven[2], E K. Bijlsma[3], A van Hagen[4], M Heijligers[5], S M. Maas[6], H E. Veenstra-Knol[7], H K. Ploos van Amstel[1], M Massink[1], Marie-Jose H. van den Boogaard[1], Aebele Mink van der Molen[1]*

[1]Wilhelmina Children's Hospital, University Medical Center, Utrecht, Netherlands, [2]Erasmus University Hospital, Rotterdam, Netherlands, [3]Leiden University Medical Centre, Leiden, Netherlands, [4]Amsterdam University Medical Centre, Amsterdam, Netherlands, [5]Maastricht University Medical Center, Maastricht, Netherlands, [6]Amsterdam University Medical Center, Amsterdam, Netherlands, [7]University Medical Center Groningen, Groningen, Netherlands.

Introduction: Cleft lip and/or palate (CL/P) are the most common craniofacial congenital malformations in humans involving various genetic and environmental risk factors and can be divided into isolated non-syndromic and syndromic forms. Many CL/P syndromes are characterized as clinically variable, genetically heterogeneous disorders, making it difficult to distinguish syndromic and non-syndromic cases. Recognition of a syndromic/genetic cause is important for personalized tailored care, including (unrecognized) co-morbidities and accurate genetic counselling. Therefore, gene panel testing is increasingly considered in the diagnostic work-up of CL/P. In this retrospective study we evaluate the yield of gene-panel CL/P testing.

Material and Methods: We included 212 CL/P cases eligible for WES-based gene panel testing between 2015 and 2020 as part of routine care. All cases were included after pre-test genetic counselling. Medical records including family history were evaluated.

Results: In 24 cases (11,3 %) causative variants underlying the CL/P were identified, including rare genetic causes requiring specific monitoring and follow-up. For example, identification of a pathogenic *KCNJ2* variant (Andersen-Tawil syndrome) led to cardiac follow-up in a CP patient and his parent, revealing a cardiac arrhythmia phenotype. Also in apparently non-syndromic cases a genetic diagnosis was made after testing. In 8 cases (3,8 %) a causative genetic diagnosis was confirmed by performing additional genetic testing, including trio WES analyses and SNP array.

Conclusions: This study exemplifies the benefit of WES- based gene panel analyses in CL/P patients in Dutch experts centres. Early diagnoses led to personalized care for patients and accurate genetic counselling of their families.

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P11.034.D Two cases of complex constitutional chromosomal rearrangements - familial implications and genetic counselling

***Diana A. Ozunu**[1], Vasilica Plăiaşu[1], Gabriela Motei[1], Mihaela Ivan[1], Adelina Dobre[2], Ana-Maria Caia-Hoanăș[2], Irina Apostol[2], Lucica Ghiță[3], Tatiana Ciomârtan[2,4]*

[1]Genetics Departament, Regional Center of Medical Genetics, National Institute for Mother and Child Care "Alessandrescu-Rusescu", Bucharest, Romania, [2]Pediatrics Departament, National Institute for Mother and Child Care "Alessandrescu-Rusescu", Bucharest, Romania, [3]Neurology Departament, National Institute for Mother and Child Care "Alessandrescu-Rusescu", Bucharest, Romania, [4]University of Medicine and Pharmacy "Carol Davila", Bucharest, Romania.

Introduction: Complex constitutional chromosomal rearrangements (CCRs) describe structural rearrangements involving at least 2 chromosomes and 3 breakpoints. Depending on their structure, CCRs can be classified as: three-way exchange, double two-way exchange and exceptional CCRs. Most double two-way translocations are de novo rearrangements, with a few familial cases reported. We present two new unrelated familial cases of imbalanced transmission for double two-way CCRs, one of maternal origin involving chromosomes 4, 5, 10 and 13, and another one of paternal origin involving chromosomes 6, 10, 12 and 18.

Materials and methods: Peripheral blood karyotyping and FISH using whole chromosome, subtelomeric and mFISH probes was applied for testing of probands and their families.

Results: Proband I is a 1 year 3 months old boy with neuromotor delay, facial dysmorphism, failure to thrive, hypospadias, behaviour problems. Conventional karyotype showed a add (10)(q26). Subsequent parental karyotype and FISH analysis described a maternal balanced CCR t(4;13)(q26-28;q22),t(5;10)(q34;q25).Proband II is a 1 month 3 weeks old boy that showed facial dysmorphism, hypospadias, pre- and postnatal growth and developmental delay. The karyotype result indicated a add(18)(q21.1). Cytogenetic investigation and FISH testing identified a paternal balanced CCR t(6;12)(p21;p13),t(10;18)(p13;q21.1).

Conclusions: Accurate characterization of CCRs by molecular cytogenetic methods is important because carriers of such rearrangements can display a wide array of phenotypes. Genetic counselling for families with CCR is difficult and should consider the fact that the risk of imbalances probably varies greatly with the nature of the rearrangement, the number of chromosomes involved and the number of breakpoints.

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P11.035.A Clinical relevance of postzygotic mosaicism in Cornelia de Lange Syndrome

*Ana Latorre-Pellicer[1], **Marta Gil-Salvador**[1], Cristina Lucia-Campos[1], Rebeca Antoñanzas-Perez[1], Laura Trujillano[2], Maria Arnedo[1], Ilaria Parenti[3], Paulino Gómez-Puertas[4], Beatriz Puisac[1], Frank J. Kaiser[5,6], Feliciano J. Ramos[1,2], Juan Pié[1]*

[1]Unit of Clinical Genetics and Functional Genomics, Department of Pharmacology-Physiology, School of Medicine, University of Zaragoza, CIBERER-GCV02 and IIS-Aragon, Zaragoza, Spain, [2]Unit of Clinical Genetics, Service of Paediatrics, University Hospital "Lozano Blesa", Zaragoza, Spain, [3]Institute of Science and Technology (IST) Austria, Klosterneuburg, Austria, [4]Molecular Modelling Group, Centro de Biología Molecular Severo Ochoa, CBMSO (CSIC-UAM), Madrid, Spain, [5]Institute for Human Genetics, University Hospital Essen, University of Duisburg-Essen, Essen, Germany, [6]Section for Functional Genetics, Institute of Human Genetics, University of Lübeck, Lubeck, Germany.

Cornelia de Lange Syndrome (CdLS, OMIM #122470, #300590, #610759, #614701, #300882) is a multisystemic genetic spectrum characterized by a recognizable craniofacial phenotype, limb malformations, intellectual disability and a wide range of other health conditions. To date, CdLS has been mainly associated with loss-of-function pathogenic variants in genes of proteins related to the cohesin complex (*NIPBL, SMC1A, SMC3, RAD21, HDAC8, BRD4,*

ANKRD11 or MAU2). Three main genetic subgroups of CdLS patients are currently recognized based on heritability: patients with hereditary pathogenic variant, with a de novo mutation (DNM) or with a post-zygotic mutation (PZM). In contrast to what is normally seen in other conditions, most of the affected individuals with mosaicism have a clinical phenotype at least as severe as those with constitutional pathogenic variants. Here we will discuss and expand the crucial role of genetic mosaicism in CdLS by reviewing a cohort of 40 CdLS patients with clinical and molecular diagnosis. We assesed the prevalence of mosaicism and present three additional patients with mosaic disease-causing variants in NIPBL. Overall, the high prevalence of mosaicism in CdLS as well as the disparity in tissue distribution should be taken into account when molecular diagnosis and familial cosegregation studies are planned.

A. Latorre-Pellicer: None. **M. Gil-Salvador:** None. **C. Lucia-Campos:** None. **R. Antoñanzas-Perez:** None. **L. Trujillano:** None. **M. Arnedo:** None. **I. Parenti:** None. **P. Gómez-Puertas:** None. **B. Puisac:** None. **F. Kaiser:** None. **F. Ramos:** None. **J. Pié:** None.

P11.036.B A functional mutation in HDAC8 gene as novel diagnostic marker for cornelia delange syndrome

Xueren Gao

Shanghai Institute for Pediatric Research, Shanghai, China.

Introduction: Cornelia de Lange Syndrome (CdLS) is a rare genetic disorder classically characterized by distinctive facies, growth retardation, intellectual disability, feeding difficulties, and multiple organ system anomalies. Previously, the diagnosis of CdLS was based mainly on identifying the typical phenotype in patients. However, with the advances in clinical molecular genetic diagnostic techniques, more patients, especially patients with milder phenotypes, are being diagnosed from detecting pathogenic mutation.

Materials and Methods: Pathogenic mutation in a female patient with a milder phenotype was detected using whole-exome sequencing, and was further characterized using bioinformatic analysis and in vitro functional experiments, including X-chromosome inactivation analysis, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and enzyme activity assay.

Results: This patient was found to harbor a novel missense mutation (c.806T>G, p.I269R) in the coding region of the HDAC8 gene, which was predicted to be pathogenic. Compared with other CdLS patients with HDAC8 mutation, the patient lacked typical facies, including synophrys and arched eyebrows. In vitro functional experiments showed the presence of skewed X-chromosome inactivation. Furthermore, the novel mutation decreased the dissolubility and enzymatic activity of HDAC8 protein.

Conclusions: The present study identified a novel missense mutation (c.806T>G, p.I269R) in the HDAC8 gene leading to CdLS, which not only provided strong evidence for diagnosis in this present patient, but also expanded the spectrum of pathogenic mutations for CdLS. The information on grants: the "National Natural Science Foundation of China" (No.81800780)

X. Gao: None.

P11.037.C Dominantly transmitted HRAS p.(Thr58Ile) pathogenic variants associated with a variable phenotype and hypertrophic cardiomyopathy in four individuals in a single family

Emily Sloper, *Oliver Murch, Arveen Kamath*

All Wales Medical Genomics Service, Cardiff, United Kingdom.

Costello syndrome (CS) is a rare condition caused by heterozygous gain-of-function germline alterations in *HRAS*. CS typically causes coarse facial features, macrocephaly, growth and developmental difficulties and skeletal, ocular and neurological problems. Cardiac features are present in the majority of individuals, with hypertrophic cardiomyopathy (HCM) occurring in approximately 2 in 3. Typically, CS arises *de novo* on the paternal allele. However, there are two previous reports of inheritance from parents with heterozygous germline *HRAS* alterations.

We present a family who share a rarely observed pathogenic variant in *HRAS* (NM_005343.2; c.173C>T, p.(Thr58Ile)). This has been reported in four other individuals previously, often in association with an attenuated phenotype. Our proband has short stature, feeding and learning difficulties, a borderline long QT and right-sided ptosis. He has distinctive features consistent with a Rasopathy, but not typical of CS. There is a variable phenotype in the family. The proband's father has longstanding HCM, a dilated aortic root and atrial fibrillation. The proband's paternal uncle has HCM and previously had a normal HCM gene panel through his Cardiology team. Cascade testing in the family has identified the c.173C>T p.(Thr58Ile) variant in the proband's father, uncle and cousin.

This report expands the phenotype caused by c.173C>T missense variants. It emphasises that *HRAS* variants should be considered in individuals with a broad phenotype, including individuals with unexplained and apparently isolated hypertrophic cardiomyopathy. It also reinforces the importance of familial testing despite the high *de novo* mutation rate, to enable appropriate access to screening, particularly cardiac screening.

E. Sloper: None. **O. Murch:** None. **A. Kamath:** None.

P11.038.D A case of moyamoya disease in a child with Costello syndrome: Expanding phenotype and proposed genotype-phenotype correlation

Norah Alsaleh[1], *Abdulaziz AlGhamdi*[2], *Amal Alhashem*[1,3], *Abdullah M Alhashem*[4], *Fowzan S Alkuraya*[5,3]

[1]*Division of Genetics and Metabolic Medicine, Department of Pediatric, Prince Sultan Military Medical City, Riyadh, Saudi Arabia,* [2]*Division of Pediatric Neurology, Department of Pediatric, Prince Sultan Military Medical City, Riyadh, Saudi Arabia,* [3]*Department of Anatomy and Cell Biology, College of Medicine, Alfaisal University, Riyadh, Saudi Arabia,* [4]*Division of Neuroradiology, Department of Radiology, Prince Sultan Military Medical City, Riyadh, Riyadh, Saudi Arabia,* [5]*Department of Translational Genomics, Center for Genomic Medicine, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia.*

Background: Costello syndrome is a rare autosomal dominant RASopathy typically caused by de novo variants in *HRAS*. It is characterized by coarse facial features, central nervous system involvement and multiple congenital anomalies including congenital heart defects and skeletal anomalies.CNS anomalies that have been reported include cerebral atrophy, ventriculomegaly, hydrocephalus and progressive posterior fossa crowding.Moyamoya disease is a progressive cerebral angiopathy characterized by bilateral internal carotid artery stenosis and abnormal collateral vessels resemble a 'puff of smoke' (moyamoya) on angiogram. Several inherited disorders have been associated with increased risk for development of moyamoya disease including the two RASopathies neurofibromatosis and Noonan syndrome. Only two cases of Costello syndrome have been reported with moyamoya disease. However, one lacked molecular confirmation and the

other had limited clinical and molecular information.Case Study: We report a 13-year-old female with history of psychomotor delay most significantly speech, focal epilepsy, congenital heart disease, myopia, thin sparse hair with brittle nails, relative macrocephaly, short stature and coarse facial features. Chromosomal microarray was normal. MRA brain revealed moyamoya disease.

Result: A previously described disease-causing variant in the *HRAS* gene consistent with the diagnosis of Costello syndrome was detected on whole exome sequencing.

Conclusion: Here we report the first case of a clinically and molecularly confirmed Costello syndrome presented with moya-moya disease. We propose a genotype-phenotype correlation related to Gly13Cys variant.

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P11.039.A Allelic heterogeneity and clinical polymorphism in patients with Cowden syndrome

Varvara A. Galkina[1], *Lyudmila A. Bessonova*[1], *Tatyana A. Vasilyeva*[1], *Rena A. Zinchenko*[1,2]

[1]*Research Centre for Medical Genetics, Moscow, Russian Federation,* [2]*N.A. Semashko National Research Institute of Public Health, Moscow, Russian Federation.*

The syndromes of multiple congenital malformations (MVP) are a large group of clinical forms of hereditary diseases. More than 3000 associated variants have been described. Currently, the concept of MVP syndromes is defined as " a stable combination of two or more non-induced by each other malformations in different systems". 4 patients from 3 unrelated families were consulted to clarify the diagnosis. Only one patient had a guiding diagnosis of Cowden syndrome. The rest of the patients had different guiding diagnoses, Sotos syndrome (patient A), Lermitt-Duclos syndrome (C), psycho-speech development disorder with convulsive syndrome (B), macrocephaly (D). Sanger sequencing revealed de novo pathogenic variant c.830C>A in exon 8 in the PTEN gene in patient D. Sequencing of "hereditary nonsyndromal mental retardation" genes panel by NGS method found mutations, c.445_450delCAAGASinsGGT in exon 5 of the PTEN gene in patient A in heterozygous state, the variant arose de novo, c.388C> in exon 5 of the PTEN gene in heterozygous state in patient B, it was inherited from his affected mother, c.59delG in exon 1 of the PTEN gene in heterozygous state in patient C, the variant arose de novo. Perhaps, allelic heterogeneity in Cowden syndrome determines the clinical polymorphism of the disease, and the molecular genetic analysis by NGS allows to clarify the diagnosis and to counsel affected families on the possibilities of prenatal diagnosis and risk of fetus pathology. Research was partially supported by RSF grant №17-15-01051 and within the state task of the Ministry of education and science of Russia.

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P11.041.C Importance of X-rays diagnostic for targeted molecular genetic analysis: Case report of rare craniometaphyseal dysplasia

Natalie Friedova[1,2,3], *Alice Baxova*[1], *Antonin Sipek Jr.*[1,2,4], *Blanka Chylikova*[1], *Renata Formankova*[5], *Eva Fronkova*[5], *Frantisek Liska*[1]

[1]*Institute of Biology and Medical Genetics, First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague, Czech Republic,* [2]*Department of Medical Genetics, Thomayer University Hospital, Prague, Czech Republic,* [3]*Internal Department, Third Faculty of Medicine and Thomayer Faculty Hospital, Charles University in Prague, Prague, Czech Republic,* [4]*Institute of Medical Genetics, Third Faculty of Medicine, Charles University in Prague, Prague, Czech Republic,* [5]*Department of Paediatric Haematology and Oncology, Second Faculty of Medicine, Charles University in Prague and Motol University Hospital, Prague, Czech Republic.*

Background: Autosomal dominant craniometaphyseal dysplasia (AD-CMD) is a rare condition defined by the occurrence of progressive diffuse hyperostosis of cranial bones with manifestation as a broad nasal bridge, widely spaced eyes, paranasal bossing and prominent mandible. Clinical diagnosis is based on radiographic findings and phenotypic features. *ANKH*is known to be the only gene associated with AD-CMD so far. We present a case of a 17-months boy with macrodolichocephaly, hypertelorism, paranasal thickening and gingival hypertrophy, coming from the physiological gravidity.

Methods: The patient was referred with a presumed diagnosis of osteopetrosis for genetic examination. Physical examination, X-ray and DNA analysis were performed. All exons and flanking intron regions (min. 10 bp) of *ANKH* were amplified by PCR and directly sequenced using Sanger method. The presence of the deletion variant was also supported by detection of a corresponding electrophoretic mobility shift using polyacrylamide gel electrophoresis.

Results: X-ray of the skull showed diffuse sclerotization in the area of facial skeleton and skull base. On X-rays of limbs club-shaped enlargement of the metaphysis of the distal femur and the proximal tibia were described. The DNA analysis showed that patient is a heterozygous carrier of the CTC deletion in exon 9 of the *ANKH* gene, resulting in a serine deletion at position 375 (rs121908406_c.1122-4delCTC_p.Ser375del). This mutation has been already described in patients with CMD.

Conclusion: We present a case report of successful molecular genetic diagnosis of rare CMD, that shows the important role of X-ray diagnosis in targeted molecular genetic diagnosis of skeletal dysplasia.

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P11.042.D Small deletion in the *CREBBP* gene detected in a fetus with short long bones, abducted thumbs and nuchal edema

Silvia Serafim[1], *Barbara Marques*[1], *Sonia Pedro*[1], *Ana R. Tarelho*[1], *Cristina Ferreira*[1], *Laurentino Simao*[1], *Cristina Alves*[1], *Marisa Silva*[1], *Jose Furtado*[1], *Sara Rangel*[1], *Ricardo Peliano*[1], *Neuza Silva*[1], *Filomena Brito*[1], *Ines Carvalho*[2], *Ana Bernardo*[2], *Alvaro Cohen*[2], *Hildeberto Correia*[1]

[1]*Instituto Nacional de Saúde Doutor Ricardo Jorge, Departamento de Genética, Unidade de Citogenética, Lisboa, Portugal,* [2]*Maternidade Alfredo da Costa, Lisboa, Portugal.*

Rubinstein-Taybi syndrome (RSTS) is an extremely rare autosomal dominant genetic disease, with an estimated prevalence of one case per 100,000-125,000 live births and it´s not usually diagnosed by prenatal ultrasound. Nevertheless, pregnancies with ultrasound abnormalities are more likely to have a fetus affected by a genetic alteration, and chromosomal microarray analysis (CMA) is still the recommended genetic test in use allowing the identification of small pathogenic deletions/duplications. Here we report a fetus referred for prenatal diagnosis due to an increased nuchal translucency in the 1st-

trimester, and nuchal edema, abducted thumbs and short long bones, in the 2nd-trimester ultrasound. Affymetrix Cytoscan 750 CMA revealed a 128 Kb interstitial deletion at 16p13.3, in a male fetus: arr[GRCh37] 16p13.3(3840720_3969211)x1. The deletion includes the first four exons of *CREBBP*. Pathogenic mutations in *CREBBP* or deletions in the 16p13.3 region including *CREBBP* are causal for RSTS. Patients with RSTS mainly exhibit distinctive facial features, broad and often angulated thumbs and halluces, intellectual disability, and postnatal growth retardation. The syndrome is almost always sporadic, and after parental analysis this deletion was shown to be de novo. After genetic counseling the parents opted to terminate the pregnancy. Even if ultrasound abnormalities not always suggest a specific syndrome, after a pathogenic CNV is identified a correlation may be possible. In this case the abducted thumbs have been previously observed in patients and the short long bones may already reflect the short stature often typical only in adulthood, suggesting it can be more common prenatally.

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P11.043.A Diet nutrition of patients with mitochondrial dysfunctions against the background of persistent microbial and viral infections

Olena Grechanina

Interregional Specialized Medical Genetic Center - Center of Rare (Orphan) Diseases, Kharkiv, Ukraine.

Purpose: to study the effect of complex treatment of mitochondrial dysfunction using cofactor-vitamin and dietary therapy, rehabilitation.

Materials and methods: over the past 10 years, 1754 patients with mitochondrial dysfunction. The genetic epidemiology of mtDNA haplotypes was studied in the population and patients with clinically established diagnosis of MtD. Among 236 examined patients, 36.5% have mitochondrial syndromes (MERRF, MELAS, NARP, Leigh,Leber, Kearns-Sayre, Fahr) and mtDNA polymorphisms (tRNA gene-lysine, mutations 8836A/G (met/val), 8472C/T(pro/leu2), 8614T/C; tRNA gene-leucine, mutations 3624A/G, 3705G/A; full sequence - mtDNA, mutations 1888G/A, 2706A/G, 8697G/A, 8860G(thr/ala), 11251A/G, 11719G/A, 11812A/G, 14687A/G, 14766C/T, 14905G/A, 15326A/G, 15452C/A, 15607A/G, 15928G/A). Persistent viral and microbial infections were detected in 73% of patients.

Results: clinical and genetic features of mtDNA polymorphism carriers were characterized by multiple organisms, clinical polymorphisms, predominant involvement of energotropic organs. It was suggested that influence of mtDNA polymorphisms on MtD expression occurs as a result of adaptive role replacement by a pathogenic one, due to altered methylation. Cofactor-vitamin and dietary therapy was used. An individual diet was developed, the nature of changes in biomarkers confirming the disorder (level of amino acids, organic acids, trace elements, carbohydrates, metals, vitamins), which provided evidence of treatment, constant monitoring of specialists. The results were highly effective (up to 83%). A new understanding of the integrated energy network of cells gives new understanding of the need for an integrated approach to restore cellular energy (D.Wolles). **Conclution**: We consider that problem solution is associated with trinity realization of genome, microbiome and virome, external environment, epigenetic status interaction.

O. Grechanina: None.

P11.044.B A rare duplication in 8p11 region

Marta Souto[1], *Pedro Botelho*[1], *Márcia Martins*[2], *Osvaldo Moutinho*[3], *Rosário Pinto Leite*[1]

[1]*Laboratório de Genética, Centro Hospitalar Trás-os-Montes e Alto Douro, Vila Real, Portugal,* [2]*Consulta de Genética, Centro Hospitalar de Trás-os-Montes e Alto Douro, Vila Real, Portugal,* [3]*Serviço de Ginecologia/Obstetrícia, Centro Hospitalar Trás-os-Montes e Alto Douro, Vila Real, Portugal.*

Introduction: The partial duplication of the short (p) arm of chromosome 8 is a rare syndrome. Clinical manifestations vary from healthy to several degrees of mental retardation, multiple congenital anomalies - like hypotonia, heart defects, brain malformations (Dandy-Walker syndrome, dilation of the third ventricle and agenesis of the corpus callosum) and facial dysmorfism. The authors presented a rare duplication involving 8p11 region. Clinical Report: 12 years old girl with mental retardation and agenesis of the corpus callosum. Cytogenetics analysis revealed extra material on the short arm of chromosome 8. Parents karyotype were normal. Fluorescence in situ hybridization (FISH) technique identified the extra material as chromosome 8. Array Comparative Genomic Hybridization (aCGH) technique revealed a duplication of 8p11.23 to 8p11.1. The segment duplicated had 6.4 Mbp and involved 62 genes. The karyotype was 46,XX,dup(8)(p11.23p11.1). arr 8p11.23p11.1 (37,348,105-43,754,516)x3.

Discussion: The present case has a 6.4Mb duplication in 8p11.23p11.1 region. The phenotypic characteristic observed in the girl included mental retardation and agenesis of the corpus callosum, which are consistent with partial trisomy 8p phenotype. There are only ten cases described with, complete or partially, 8p11.23p11.1 region duplication, most of them higher than 20Mb and consequently with more severe clinical manifestations.

Conclusion: Every new case of a rare chromosomal alteration should be reported in order to obtain a more precise genotype/phenotype correlation, improving risk evaluation and genetic counselling.

M. Souto: None. **P. Botelho:** None. **M. Martins:** None. **O. Moutinho:** None. **R. Pinto Leite:** None.

P11.045.C New case of Dyskeratosis congenita 4 mimicking Hoyeraal-Hreidarsson syndrome with novel *TERT* gene mutation

Ece Çepni[1], *Sahin Avci*[2], *N. Bilge Satkın*[2], *Hülya Kayserili*[1,2,3]

[1]*Institute of Health Sciences, Koç University School of Medicine, Istanbul, Turkey,* [2]*Diagnostic Center for Genetic Diseases, Koç University Hospital, Istanbul, Turkey,* [3]*Medical Genetics Department, Koç University School of Medicine, Istanbul, Turkey.*

Dyskeratosis congenita(DC) is a rare, multisystemic telomere biology disorder classically characterized by a triad of mucocutaneous features: abnormal skin pigmentation, oral leucoplakia, nail dysplasia; accompanied by various somatic findings. Bone marrow failure, pulmonary disease and predisposition to malignancy are the primary causes of mortality. We here report a consanguineous DC family, displaying autosomal recessive inheritance. Index, 9-month-old girl, was consulted due to growth retardation of antenatal onset with delayed achievement of developmental

milestones. She had microcephaly, hypoplastic labia minora, mild facial dysmorphism comprising bitemporal narrowing, strabismus, prognathism(mild), prominent nasal root(mild), smooth philtrum and low-set ears. The family had undergone genetic counseling but deferred molecular testing at that moment. At 22 months, she presented immunodeficiency, complicated with recurrent infections/intractable diarrhea and anemia. Physical examination showed oral leukoplakia, thin/sparse hair, lacy reticular hyperpigmentation on left axillary region and right hemithorax; cranial MRI showed cerebellar hypoplasia. WES revealed a homozygous missense variant in the reverse transcriptase domain of the *TERT* gene. Short relative telomere length(TL), measured by flow-FISH, was compatible with infancy-onset short telomere syndrome. Detailed pedigree analysis showed no clinical evidence of DC except premature hair graying, anemia and cancer in blood relatives across three generations. Identification of heterozygous *TERT* mutation and short TL in extended family members, segregating with the phenotype, highlighted disease anticipation and further confirmed the diagnosis of DC. Our findings expand the genotype-phenotype correlation of DC; thus underline the importance of integrating clinical information, molecular data and TL to facilitate the recognition of the etiopathogenesis of telomere syndromes.

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P11.046.D Detection of 3p25 microdeletion syndrome in the Macedonian patient with significant psychomotor retardation

Violeta Anastasovska[1], *Elena Sukarova-Angelovska*[1], *Dragica Nestoroska*[1], *Nikolina Zdraveska*[2], *Gordana Ilieva*[1], *Milica Pesevsa*[1], *Ivana Stojanova*[3]

[1]*Genetic Laboratory, University Clinic for Pediatrics, Ss. Cyril and Methodius University in Skopje, Faculty of Medicine, Skopje, Macedonia, The Former Yugoslav Republic of,* [2]*Department of Neonatology, University Clinic for Pediatrics, Ss. Cyril and Methodius University in Skopje, Faculty of Medicine, Skopje, Macedonia, The Former Yugoslav Republic of,* [3]*In vitro Fertilization and Andrology Laboratory, University Clinic of Gynecology and Obstetrics, Ss. Cyril and Methodius University in Skopje, Faculty of Medicine, Skopje, Macedonia, The Former Yugoslav Republic of.*

Introduction: The array Comparative Genomic Hybridization (aCGH) is a first tier diagnostic tool for detection of submicroscopic genomic changes and pathogenic copy number variants (CNVs) in the patients with pathological conditions and wideranging phenotypes.

Materials and Methods: aCGH was performed in a 7.5-year-old female Macedonian patient with clinical signs of dysmorphia and significant developmental delay using the Affymetrix® CytoScan™ 750K Array (Applied Biosystems), that comprises 550 k non-polymorphic and 200 k SNP markers. The data was analysed using Chromosome Analysis Suite (ChAS) Software (v4.0).

Results: The child was referred for further evaluation on 8th postnatal day because of dysmorphic features. She was born small for gestational age, after 39 weeks of gestation with a birth weight of 2340g and birth length of 45cm. Hypertelorism and antimongoloid eye slant, micrognathism, webbed neck, pyelonidal cyst and preaxial polydactily both on the left foot and right hand were noticed. During the follow up the child had several hospitalizations because of failure to thrive requiring a tube feeding, anemia and significant psychomotor retardation. The karyotype was normal (46,XX). aCGH analysis showed deletion of 3,243 segment on 3p25.3 chromosome (30 OMIM genes) classified as pathogenic according to aDGV, ClinVar and OMIM databases. The genes, SETD5, SLC6A1 and SLC6A11, have been proposed as the main candidates that when deleted contribute to the key features associated with 3p25 deletion syndrome.

Conclusions: The aCGH analysis as a widely accepted tool that supplements conventional karyotyping allowed genetic diagnosis of our patient with significant psychomotor retardation.

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P11.047.A Evaluation of the diagnostic rate in children with dysmorphic features - one genetic center experience

Milena Stoyanova[1,2], *Mari Hachmeriyan*[1,2], *Mariya Levkova*[1,2], *Valentina Miteva*[1,2], *Lyudmila Angelova*[1]

[1]*Medical University, Varna, Bulgaria,* [2]*University Hospital Saint Marina, Varna, Bulgaria.*

Introduction: Dysmorphic features/multiple congenital anomalies in children are common indication for genetic counseling. In some patients the cause is a recognizable syndrome, but in most cases the initial diagnosis is unclear, the process is time-consuming and difficult. Moreover, the condition often cannot be confirmed etiologically. The aim of the study is to summarize our experience in establishing the diagnoses in dysmorphic children.

Materials and methods: The study includes 706 pediatric patients (0-18 years), referred to the Genetics Unit of the University Hospital Saint Marina, Varna for a period of five years (2015-2019). Clinical phenotyping, imaging examinations, appropriate genetic and metabolic investigations were offered to children with dysmorphic features/ multiple congenital anomalies. Specialized computer programs/dysmorphology databases were applied.

Results: 336 out of 706 (47.5%) consulted children (mean age 3.9 years) with multiple congenital anomalies with or without developmental delay were suspected of malformative/dysmorphic syndrome. Karyotyping, molecular genetic or metabolic tests were performed in 306 (92%) children (≥ 2 genetic tests were appropriately applied to 104 patients). Based on these analyses, 121(36%) children were genetically diagnosed: 70 patients (25%) with chromosomal pathology, 32 (9.5%) with single-gene pathology and 19 (5.6%) with microdeletion/microduplication disorder. Other 41(12%) children were clinically diagnosed based on specific phenotype.

Conclusion: The role of the medical geneticist in achieving an accurate diagnosis among children with dysmorphic features is essential. Once confirmed, it could affect the disease management, as well as provide more personalized approach and contribute families with proper evaluation of the recurrence risk.

M. Stoyanova: None. **M. Hachmeriyan:** None. **M. Levkova:** None. **V. Miteva:** None. **L. Angelova:** None.

P11.048.B A new heterozygous c.730T>A, p.(Cys244Ser) variant in *TP63* associated with severe hydronephrosis and volar nails

Mariana Tomásio Neves, *Patricia Dias, Ana B. Sousa*

CHULN - Hospital de Santa Maria, Lisbon, Portugal.

Introduction: *TP63*-related pathologies are a group of autosomal dominant phenotypes with variable features of ectodermal dysplasia, distal limb malformations/dysplasia and lip/palate clefts. These can occur as distinct syndromes (AEC, ADULT, EEC3, LMS) or has isolated malformations (split-hand/foot malformation and

isolated cleft lip/ palate). Although these conditions exhibit variable expressivity, incomplete penetrance and clinical overlap, some genotype-phenotype correlations have been described. We report a case of EEC3 syndrome with minor hand/foot anomalies and severe prenatal hydronephrosis associated with a new *TP63* variant. Case report: A 3-month-old girl, who was diagnosed with severe bilateral hydronephrosis at 15 weeks of gestation, presented post-natally with cleft palate, bilateral volar nails of the fifth finger and second toe and lacrimal duct obstruction. Left ureterostomy and right ureter endoscopic dilation were performed at 2 months. On follow-up, subtle ectodermal dysplasia was noticed. Sequencing of *TP63* (NM_003722.4) identified a heterozygous c.730T>A, p.(Cys244Ser) variant, located in a mutational hot-spot in the DNA binding domain.

Discussion: This patient's phenotype is best classified as EEC3 syndrome. Volar nails are a peculiar minor anomaly and constitute an important clue to this specific diagnosis. This case highlights the association of severe congenital genitourinary malformations with EEC3 syndrome, a feature not described in other *TP63*-related pathologies. Several patients carrying a variant in the adjacent 243 codon are published presenting EEC3 (predominantly) and ADULT phenotypes. On follow-up, it will be interesting to check whether this patient develops features of ADULT syndrome, namely breast hypoplasia and skin freckling.

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P11.049.C Mandibulofacial Dysostosis with Microcephaly due to EFTUD2 gene mutation. Expanding phenotypic spectrum with first prenatal case reported

Sofia Ourani[1], *Elisabeth Gabau*[2], *Nuria Capdevilla*[2], *Miriam Guitart*[3], *Neus Baena*[3], *Anna Ruiz*[3]

[1]*Pediatric Neurology, Hospital Vall d´Hebron, Barcelona, Spain,* [2]*Paediatric Unit, Parc Taulí Hospital Universitari, Institut d'Investigació i Innovació Parc Taulí I3PT, Universitat Autònoma de Barcelona, Sabadell, Barcelona, Spain,* [3]*Genetics Laboratory, UDIAT-Centre Diagnòstic, Parc Taulí Hospital Universitari, Institut d'Investigació i Innovació Parc Taulí I3PT, Universitat Autònoma de Barcelona, Sabadell, Barcelona, Spain.*

Introduction: Mandibulofacial dysostosis with microcephaly (MFDM) is a multiple malformation syndrome due to haploinsufficiency of EFTUD2 gene. Major clinical characteristics include malar and mandibular hypoplasia, variable intellectual disability, external ear malformations, and hearing loss mainly conductive. Associated craniofacial malformations include cleft palate, choanal atresia, zygomatic arch cleft and facial asymmetry. Other findings reported are cardiac anomalies, thumb anomalies, esophageal atresia, short stature, spine anomalies.

Material and methods: We present the phenotype and genetic findings of three unrelated patients with MFDM confirmed by EFTUD2 mutations. Case 1: girl of healthy nonconsanguineous parents delivered at 35+5weeks of gestation and diagnosed with esophageal atresia type I, macroglossia, hemifacial microsomia, cleft palate, muscular ventricular septal defect, severe global developmental delay. Case 2: eight month toddler asked for genetic evaluation for antecedents of prenatal polyhydramnios, esophageal atresia type III, cleft palate and moderate developmental delay. Case 3: fetus of pregnancy interrupted at 19 +4weeks of gestational age for ultrasound findings of hydrocephalus, cerebellum hypoplasia, retrognathia, lumbar lordosis, varus feet. Autopsy confirmed ecographic findings and additionally revealed microstomia, tongue agenesis and fusion of five thoracic vertebras, corpus callosum agenesis, esophageal atresia type III. Three different pathogenic mutations in EFTUD2 gene were found, in cases 1 and 2 through targeted gene sequence and in case 3 through exome sequence.

Conclusion: Expanding the clinical heterogeneity of MFDM and its connection with major congenital defects. Additionally, we report the first prenatal case diagnosed with the disease and clinical findings not yet described in the phenotypic spectrum.

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P11.050.D High prevalence of gene dosage anomalies in patients with Ellis Van Creveld syndrome

FRANCESCA PICECI-SPARASCIO[1,2], *Isabella Torrente*[1], *Maria Cecilia D'Asdia*[1], *Valentina Guida*[1], *Federica Consoli*[1], *Annamaria Onori*[1], *Barbara Torres*[1], *Laura Bernardini*[1], *Tommaso Mazza*[3], *Paolo Versacci*[4], *Maria Cristina DiGilio*[5], *Bruno Marino*[4], *Alessandro De Luca*[1]

[1]*Medical Genetics Division, Fondazione IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy,* [2]*Department of Experimental Medicine, Sapienza University, Roma, Italy,* [3]*Laboratory of Bioinformatics, Fondazione Casa Sollievo della Sofferenza, IRCCS, San Giovanni Rotondo, Italy,* [4]*Department of Pediatrics, Sapienza University, ROMA, Italy,* [5]*Medical Genetics Unit, Bambino Gesù Children's Hospital and Research Institute, IRCCS, ROMA, Italy.*

Ellis-Van Creveld syndrome (EvC) is a rare autosomal recessive skeletal ciliopathy presenting with postaxial polydactyly, ectodermal dysplasia and congenital heart disease. EvC is due to biallelic mutations in *EVC* or *EVC2*. We scanned for mutations by Next-Generation-Sequencing (NGS) the *EVC* and *EVC2* genes in a cohort of 99 suspected EvC individuals. Analysis identified the cause of the disease in 49 patients (49%). 33/49 (67%) were mutated in *EVC* and 16/49 (33%) in *EVC2*. Mutation-negative patients underwent NGS analysis using an extended ciliary targeted panel. Analysis allowed to genetically-characterizing additional 14 patients, found to be mutated in ciliary genes related to skeletal ciliopathies other than EvC. To search for gene-dosage anomalies, the remaining 36 mutation-negative patients were further tested by Multiplex Ligation-Dependent Probe Amplification (MLPA), Chromosomal Microarray (CMA), or both. MLPA/CMA analysis identified compound heterozygous or homozygous deletions in 9 patients. In particular, 6 had deletions in *EVC* and 3 in *EVC2*. Of note, two *EVC* recurrent deletions were identified, affecting 4 and two patients respectively. Present study shows that gene-dosage anomalies represent a significant proportion (approximately 15%) of the *EVC*/*EVC2* mutation spectrum. Considering the clinical overlapping between skeletal ciliopathies, and the prevalence of *EVC*/*EVC2* gene-dosage anomalies, we recommend a two-tier diagnostic approach integrating a primary search for point mutations by a ciliary targeted NGS analysis, followed by a quantitative assay (MLPA/CMA), waiting for reliable pipelines for the detection of intragenic CNVs throughout NGS technologies.This research was funded by the Italian Ministry of Health, grant numbers RC2019/ RC2020

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P11.051.A A case of ESCO2 spectrum disorder without limb reduction defects

Sofía M. Siccha-Arancibia[1,2], *María Palomares-Bralo*[1,3], *Marta Pacio-Miguez*[1], *Gloria Lopez-Sobrino*[2], *Carmen Rodríguez-Jimenez*[1],

Ángela del Pozo[1,3], Elena Mansilla[1,3,4], Fé García-Santiago[1,3,4], Sixto García-Miñaúr[1,3], Fernando Santos-Simarro[1,3,4]

[1]Institute of Medical & Molecular Genetics (INGEMM)-IdiPAZ, Hospital Universitario La Paz, Madrid, Spain, [2]Department of Pediatrics, Hospital Universitario la Paz, Madrid, Spain, [3]Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER, U753), Instituto Carlos III, Madrid, Spain, [4]Skeletal Dysplasia Multidisciplinary Unit (UMDE) and ERN BOND, Hospital Universitario La Paz, Madrid, Spain.

Introduction: Roberts syndrome (MIM 268300) at the severe end and SC phocomelia syndrome (MIM 269000) at the mildest end belong to the RBS/SC or ESCO2 (establishment of cohesion1 homolog 2) spectrum disorder, a rare limb reduction defect syndrome, caused by biallelic pathogenic variants in the *ESCO2* gene. *ESCO2* encodes an essential protein that establishes the sister chromatid cohesion during S phase, therefore the premature centromere separation (PCS)/heterochromatin repulsion (HR) is a typical cytogenetic anomaly of the condition. Mortality is high among the severely affected patients, while SC phocomelia individuals usually survive to adulthood. Phocomelia is universally described. Interestingly, there is one case published, with mild clinical features and no limb reduction abnormalities (Gogh et al., 2010). We report a case of an 8-year-old girl with mild dysmorphic facial features and no major structural defects.

Materials and Methods: We performed a NGS custom panel containing 1663 genes involved in common genetic disorders (RD seq (R) v6.0), and a karyotype using G- and C-banding techniques.

Results: Our patient shows limb rhizomelic shortening and a progressive brain leukopathy with normal neurologic examination. We found a homozygous *ESCO2* variant (NM_001017420.2: c.307_311del;p.Lys103Glufs*2), classified as pathogenic, following ACMG guidelines (Richards et al., 2015). Her consanguineous parents were both heterozygous for the variant. The patient's karyotype confirmed premature centromere separation (PCS)/ heterochromatin repulsion (HR).

Conclusions: Our case supports a wider clinical spectrum of manifestations in RBS/SC spectrum, highlighting that phocomelia might not be universally present. In addition, it illustrates the importance of cytogenetics in the diagnosis of this disorder.

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P11.052.B Getting the most out of Exome sequencing data

***Javier López**, María José Roca, Laura González, Daniel Trujillano, Cristina Camprubí, Irina Royo, Xavier Pinasa, Luisa Aparicio, Raquel Nava, Albert Torrents*

Reference Laboratory, Barcelona, Spain.

Introduction: Exome sequencing (WES) currently is a solid diagnostic tool for heterogeneous genetic diseases. However, a partial and insufficient analysis of WES data could lead to misdiagnoses. We compiled 13 cases with previous negative WES results, in which a thorough reanalysis of the data yielded new candidate genes with diagnostic potential.

Materials-and-Methods: Our cohort included 300 patients with syndromic neurodevelopmental abnormalities. WES analyses were guided by HPO terms and custom gene panels based on literature. For those inconclusive results, a secondary and more objective inspection was performed.

Results: Based on thorough reanalysis of existing WES data, we identified six variants of unknown significance (VUS) in *PHF12, SYNPO2L, KCNJ16* and *XKR6* genes that could explain the patients´ phenotype; three pathogenic variants in *SCN2A*, *DPYS* and *MVK* related to phenotypes not described for them until now; two pathogenic variants in *BBS1/ALG8* and *POGZ/TRIO* genes that could explain a particular mixed phenotype of two patients. Finally, we report five novel pathogenic variants in *SMARCA2, MECP2, KRT14, NOTCH3* and *SHANK3*, not present in any database.

Conclusions: The results highlight the clinical diagnostics potential of a thorough reanalysis of previously negative WES cases. Based on the identification of new candidate genes, the increase of phenotypic spectrum of the diseases diagnosed (even reporting cases with a dual diagnosis) and the finding of novel pathogenic variants, in 5% of cases, we recommend going beyond the basic guided genetic analysis for HPO terms, especially in cases with a strong suspicion of an underlying genetic disease and a previous inconclusive WES result.

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P11.054.D The Wales Infants' and childreN's Genome Service' (WINGS): Diagnostic rapid whole genome sequencing for unwell children with a suspected rare genetic diagnosis

*Emily Sloper, **Oliver Murch**, Jana Jezkova, Megan Fealey, Joseph Halstead, Thomas Stoneman, Elle McNeil, Angharad Williams, Michelle Wood, Katherine Burke, Siva Oruganti, Jennifer Calvert, Hywel Williams, Caroline Pottinger, Francis H. Sansbury, Sian M. Morgan, Sian Corrin*

All Wales Medical Genomics Service, Cardiff, United Kingdom.

A significant proportion of unwell neonates and children have an underlying, rare genetic diagnosis. Rapid whole genome sequencing (rWGS) has a positive impact on care by reducing the need for multiple diagnostic tests and facilitating treatment decisions. It may also reduce the length of time infants and children require intensive care and prevent repeat inpatient admissions.

In 2019, the All Wales Medical Genomics Service formed a multidisciplinary working group to establish a rWGS service for acutely unwell infants and children. The group consisted of intensive care clinicians, geneticists and laboratory staff. A diagnostic pipeline was developed using previous research results and laboratory testing procedures were validated. Variants are interpreted and reported by applying the latest ACGS/ACMG guidelines. In April 2020, the 'Wales Infants' and childreN's Genome Service' (WINGS) was launched.

WINGS is the first commissioned, diagnostic rWGS service for acutely unwell children in the UK National Health Service. Patients are eligible if a monogenic cause for their illness is suspected, a trio structure is available, and a timely genetic diagnosis might alter clinical management.

Fourteen families have completed testing to date. Pathogenic or likely pathogenic variants were identified in 5 probands. Additionally, a 'hot' variant of uncertain significance in a candidate gene was reported in another patient. Mean time to reporting was 11 calendar days (range 6-26 days).

In summary, we have introduced the UK's first national diagnostic rWGS service for acutely unwell children. We will present early service outcomes and the impact from a laboratory and clinical perspective.

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P11.055.A Full penetrance of craniofacial midline traits in a large multigenerational family with a nonsense *GLI2* variant and highly variable phenotypic expression

Francisco J. Rodríguez-Contreras[1,2], *Purificación Ros-Pérez*[3], *Fe A. García Santiago*[1], *Elena Vallespín*[1,4], *Ángela Del Pozo*[1,5], *Mario Solís-López*[1], *Angel Campos-Barros*[1,4]

[1]*INGEMM, IdiPAZ, Hospital Universitario La Paz, Madrid, Spain,* [2]*Centro de Salud Galapagar, Galapagar, Madrid, Spain,* [3]*Hospital Universitario Puerta de Hierro, Majadahonda, Madrid, Spain,* [4]*CIBER de Enfermedades Raras (U753), ISCIII, Madrid, Spain,* [5]*CIBER de Enfermedades Raras (U753), Madrid, Spain.*

GLI2 encodes a transcription factor involved in the Sonic Hedgehog signaling pathway which plays a key role in pituitary development. Loss of function pathogenic *GLI2* variants have been implicated in the etiology of congenital hypopituitarism spectrum and Culler-Jones syndrome (MIM#615849) with incomplete penetrance and a highly variable clinical expression. *Aim:* To identify common phenotypic traits, especially midline craniofacial anomalies (hypotelorism and palate alterations), in a family with multiple members presenting with a pathogenic heterozygous nonsense *GLI2* variant, NM_005270.4:c.3676C>T, p.(Arg1226*), detected in a proband with combined pituitary hormone deficiency (CPHD).

Methods: Targeted NGS analysis (custom panel HIPOPIT_V3; 310 genes) of three generations family members (n = 12). Phenotypic evaluation by physical examination and/or medical records review. Evaluation of internal, external and interpupillary interchantal distances (by transparent ruler and comparison with reference tables) and palate (by direct visual examination and on images).

Results: 8 out of 12 family members presented the pathogenic *GLI2* variant. Although they presented with highly variable phenotypic features, including holoprosencephaly (unborn fetus), CPHD (index case), postaxial polydactyly (grandfather, granduncle), syndactyly (proband sisters), or congenital renal dysplasias, all 8 shared common midline craniofacial defects, such as decreased inner- and/or outer interchantal distance and high arched or narrow palate.

Conclusions: Loss-of-function *GLI2* mutations are characterized by highly variable expressivity, which suggests the oligomeric contribution of additional genetic determinants. In contrast to phenotypic traits such as polydactyly and hypopituitarism, which show incomplete penetrance, midline craniofacial anomalies seem to segregate with complete penetrance, with variable severity, among the examined family members. GRANT: PI18/00402 ISCIII

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P11.056.B Familial Grange syndrome: 1st case report of *YY1AP1* homozygous deletion

Eléonore Viora-Dupont[1], *Anne-Sophie Denommé-Pichon*[1,2], *Martin Chevarin*[2], *Olivier Patat*[3], *Marjolaine Willems*[4], *Juliette Albuisson*[1], *Ange-Line Bruel*[2], *Marion Aubert-Mucca*[3], *Michel Galinier*[5,6,7], *Romain Itier*[5], *Amélie Piton*[8], *Bénédicte Gerard*[9], *Patrick Callier*[2], *Christel Thauvin*[1,2], *Christophe Philippe*[2], *Laurence Faivre*[1,2], *Antonio Vitobello*[2]

[1]*Genetics Department and Reference Center for Developmental Disorders and Malformative Syndromes for East France, Dijon University Hospital, Dijon, France,* [2]*Inserm UMR 1231 GAD, Faculty of Health Sciences, University of Burgundy and Franche-Comté, Dijon, France,* [3]*Service de Génétique Médicale, CHU Toulouse, France, Toulouse, France,* [4]*Département de Génétique Médicale, Maladies Rares et Médecine Personnalisée, Univer Montpellier, CHU de Montpellier, CLAD ASOOR Montpellier, Montpellier, France,* [5]*Fédération des Services de Cardiologie, CHU Toulouse-Rangueil, Toulouse, France,* [6]*UMR UT3 CNRS 5288 Evolutionary Medicine, Obesity and heart failure: molecular and clinical investigations. INI-CRCT F-CRIN, GREAT Networks, Toulouse, France,* [7]*Université Paul Sabatier-Toulouse III; Faculté de Médecine, Toulouse, France,* [8]*Unité de Génétique Moléculaire, Strasbourg University Hospital, Strasbourg, France,* [9]*Laboratoire de Diagnostic Génétique, Institut de Génétique Médicale d'Alsace, Hôpitaux Universitaires de Strasbourg, Strasbourg, France.*

Grange syndrome (GRNG - MIM#135580) is a rare recessive disorder associating variable features including diffuse vascular stenoses, brachysyndactyly and osteopenia with increased bone fragility. Some individuals also present with cardiac malformations as well as mild to moderate mental retardation. Since its first description in 1998, only 12 individuals from 7 families have been reported, carrying homozygous or compound heterozygous frameshift or nonsense variants in *YY1AP1*.

We performed exome sequencing in a patient presenting with cutaneous and bone syndactyly and negative array-CGH and limb malformations panel results. After a hemorrhagic stroke at the age of 16 months, a clinical diagnosis of GRNG was hypothesized. Copy number variant analysis from exome data, identified a homozygous intragenic non in-frame deletion of 1.84 Kb encompassing exons 8 and 9 of *YY1AP1* confirming a molecular diagnosis of GRNG. Genetic data revealed several regions of homozygosity indicating a possible consanguinity. The research of small intragenic *YY1AP1* deletions in our local database identified the same homozygous variant in another individual presenting with cutaneous syndactyly, cardiac malformation and intellectual disability and referred for an unspecific malformative syndrome. Familial analysis revealed a close relatedness between the two individuals and the identification of additional members of the family compatible with GRNG.

Here, we describe the first familiar case of GRNG caused by a small homozygous intragenic deletion in *YY1AP1*. Taken together, our results advocate the interest in applying exome or genome sequencing to detect causative variants not identifiable by other methods in heterogeneous malformative genetic disorders.

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P11.057.C H3.3 variants: from cancer to neurodevelopmental disorders

Fernando Santos-Simarro[1,2,3], *Marta Pacio-Míguez*[1], *Ángela del Pozo*[1,2], *Carmen Jiménez Rodríguez*[1], *Cristina Cruz Castellanos*[4], *Mario Solís*[1], *Victoria Eugenia F. Montaño*[1], *María Victoria Gomez del Pozo*[1], *Natividad Gallego Onís*[1], *María Esther Rubio Martín*[1], *Pablo Lapunzina*[1,2,3], *María Palomares-Bralo*[1,2,3], *Sixto García-Miñaúr*[1,2,3]

[1]Instituto de Genética Médica y Molecular (INGEMM) Hospital Universitario La Paz, Madrid, Spain, [2]CIBERER, Centro de Investigación Biomédica en Red de Enfermedades Raras, ISCIII, Madrid, Spain, [3]European Reference Network, ERN ITHACA, Madrid, Spain, [4]Servicio de Oncología Médica, Hospital Universitario La Paz, Madrid, Spain.

Introduction: In the past years, somatic mutations affecting *H3F3A* and *H3F3B* genes coding for Histone 3 variant H3.3 have been established as well-known drivers for tumour development. In addition, de novo germline missense variants in these genes have been recently associated with a novel neurodegenerative disorder apparently not related with cancer development. However, the complete phenotypical impact of these germline mutations remains unknown. Here we describe the first case carrying a *H3F3B* de novo germline variant showing the neurological phenotype associated with lung carcinoma.

Materials and methods: We performed a WES study through a trio approach (SureSelect Human All Exon V6 technology) in a HiSeq 4000 platform (Illumina, San Diego, CA).

Results: A de novo, heterozygous, germline missense variant in *H3F3B* NM_005324.4:c.71A>G p.(Lys24Arg) was identified in a 36 year-old woman presenting with severe intellectual disability, dysmorphic facial features and congenital anomalies. Interestingly, she developed a typical carcinoid tumour of the pulmonary middle lobe at the age of 32, although the patient did not report toxic habits or a family history of cancer.

Conclusions: Given the well-known role of H3.3 somatic mutations in oncogenesis, the description of this new case with an *H3F3B* germline variant and an unusual form of cancer, should prompt us to investigate and follow up these patients accordingly in order to try to ascertain the precise role of germline H3.3 variants in cancer predisposition. MPM is supported by the Raregenomics network (S2017/BMD-3721). This work is partially funded by the ISCIII, MICINN (PI19/01681) and the European Social Fund.

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P11.058.D Novel CNS malformations in De Barsy syndrome A

Hanem S. Abdelraouf, *Amal M. Alhashem*

Prince Sultan Military Medical City, Riyadh, Saudi Arabia.

Title: Novel CNS malformations in De Barsy syndrome A.Author: Abdelraouf Hanem[1],Alhashem Amal[1](1)Genetic Division, pediatric Department, Prince Sultan Military Medical City, Riyadh, SaudiArabiaIntroduction: De Barsy syndrome was first reported in 1968. The disorder is now classified asautosomal recessive cutis laxa type3A.This disorder involves a wide spectrum of symptoms andsigns that result from defects in connective tissue. Most cases are characterized by cutis laxa, aprogeria-like appearance, and ophthalmologic abnormalities.

Method: Observational studyResult: A full term baby with intrauterine growth retardation.He has dysmorphic facial features; hypertelorism, large low set ears, thin lips, and prominent hairy forehead.He has the classic picture of cutis laxa type IIIA in the form of bilateral corneal clouding,strabismus, bilateral inguinal hernia,micropenis,hypotonia,hyperreflexia, clonus, multiplejoints hypermobility,adducted thumbs with fixed extension of inter phalangeal joint of thethumb and clenched hands, abnormal fat pad at buttocks and upper thighs,and developmentaldelay. The patient has laryngomalacia and brain malformations ;cerebellar atrophic changes,hypoplastic pons, vascular tortuosity and elongation of the vessels of the circle of Willis withaneurysmal dilatation,and Short dysgenic corpus callosum. Baby has abnormal lipid profile.Genetic testing done showed homozygous variant in *ALDH18A1*(AR cutis laxa type III)in additionto heterozygous variant in *LDLR* (AD familial hypercholesterolemia).

Conclusion: phenotypic features of De Barsy syndrome A include CNS malformation asCerebellar atrophic changes, hypoplastic pons, vascular tortuosity and elongation of the vesselsof the circle of Willis,and corpus callosum malformation.

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P11.059.A Detection of a rare ADNP variant causing Helsmoortel-van der Aa syndrome: A Case Report

Boban Dobrevski[1], *Aleksandar Petlichkovski*[1], *Meri Kirijas*[1], *Gorjan Milanovski*[1], *Todor Arsov*[1], *Teodora Brnjarchevska Blazevska*[1], *Olivija Efinska-Mladenovska*[1], *Olgica Sibinovska*[1], *Elena Shukarova Angelovska*[2]

[1]*Ss. Cyril and Methodius University in Skopje, Faculty of Medicine-Skopje, Institute of Immunobiology and Human Genetics, Skopje, Macedonia, The Former Yugoslav Republic of,* [2]*Ss. Cyril and Methodius University in Skopje, Faculty of Medicine-Skopje, PHI University Clinic for child diseases, Skopje, Macedonia, The Former Yugoslav Republic of.*

Introduction: Helsmoortel-van der Aa syndrome, or Intellectual Disability Autosomal Dominant 28 (MDR28, OMIM 615873) is a rare neurodevelopmental genetic disorder affecting about 1-9/100 000 individuals (0.17% of patients with intellectual disability and autism). The broad and variable spectrum of clinical manifestations includes global developmental delay, autistic features and neuropsychiatric or behavioral issues, seizures and distinct dysmorphic facial features. We report a case of a nine-month old girl with delayed motor development (delays in sitting and holding head up), general hypotonia, dysmorphic facial features (prominent forehead, discrete strabismus) and noticeable happy demeanor.

Materials and Methods: The initial metabolic screen and array-CGH results were normal and the additional genetic testing included an NGS gene panel of 37 spinal muscular atrophy associated genes (AARS1, ASAH1, ASCC1, ATP7A, BICD2, BSCL2, CHCHD10, DCTN1, DNAJB2, DYNC1H1, EMILIN1, EXOSC3, EXOSC8, FBXO38, GARS1, HEXA, HSPB1, HSPB3, HSPB8, IGHMBP2, LAS1L, PLEKHG5, RBM7, REEP1, SCO2, SETX, SIGMAR1, SLC25A21, SLC5A7, SPTAN1, SYT2, TRIP4, TRPV4, UBA1, VAPB, VRK1, WARS1) including ADNP using Illumina NGS platform, SMN1/SMN2 deletion/duplication by MLPA and AR-repeat by PCR analysis.

Results: The genetic testing identified a heterozygous pathogenic ADNP frameshift variant c.539_542delTTAG, p.Val180-Glyfs*17, not present in the databases of human genetic variation (gnomAD) and reported as pathogenic in 6 other cases (ClinVar). The variant is predicted to create a premature stop codon and protein truncation and results in a loss-of function of ADNP, affecting chromatin packing and transcription.

Conclusion: New sequencing technologies, such as gene panel testing, allow early diagnosis and better management of rare genetic diseases.

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P11.060.B *HHAT*-related multiple congenital anomalies: Report of an additional family and delineation of the syndrome

*Shruti Pande[1], Periyasamy Radhakrishnan[1], Naveenchandra M. Shetty[2], Anju Shukla[1], **Katta M. Girisha**[1]*

[1]Kasturba Medical College, Manipal, Udupi, India, [2]Mediscan Diagnostic Centre, Mangalore, Manglore, India.

Introduction: *HHAT* (Hedgehog acyl-transferase) mediates the post-translational modification of downstream proteins in the hedgehog (Hh) signalling pathway. There are two reports from two unrelated families with sequence variants in *HHAT*. We hereby report the third family with three affected conceptuses.

Methods: We performed clinical evaluation of a seven years female child born to a consanguineous couple who also had two other affected pregnancies. We did chromosomal analysis followed by exome sequencing for the living proband and her parents.

Results: The proband had severe microphthalmia, microcephaly, skeletal dysplasia (bell-shaped thorax, short and angel shaped epiphyses of hands and feet), midface retrusion, short columella, depressed nasal bridge, everted lower lip, and a single central incisor. Her karyotype is 46, XY. Exome sequencing revealed a novel biallelic in-frame deletion, c.365_367del; (p.Thr122del) in exon 5 of *HHAT* (NM_001122834.3). Additionally, in this family, one of the affected fetuses had alobar holoprosencephaly. The key features of the HHAT-related multiple congenital anomaly syndrome are anophthalmia/microphthalmia, short stature, skeletal dysplasia, microcephaly, holoprosencephaly, cerebellar vermis hypoplasia and sex reversal.

Conclusion: We describe the third family with multiple malformations in three conceptuses with identification of the biallelic variant c.365_367del; (p.Thr122del) in exon 5 of *HHAT* in the living proband. It appears that the defective functioning of *HHAT* affects the downstream proteins in the pathway including the Sonic (SHH), Indian (IHH), and Desert (DHH), the alterations in which are associated with abnormalities of the eyes, face, nervous system, skeleton and the genitourinary system during the embryonic development.

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P11.061.C Variant in *HNRNPR* leading to developmental delay with facial dysmorphism and bone abnormalities: a case report

Marie Massier[1], *Marta Spodenkiewicz[1], Nathalie Bednarek[2], Boris Keren[3], Emilie Landais[1], Lucas Hérissant[1], Céline Poirsier-Violle[1], Martine Doco-Fenzy[1]*

[1]Department of Genetics, Reims University Hospital, Reims, France, [2]Department of Pediatrics, Reims University Hospital, Reims, France, [3]Department of Genetics, La Pitié-Salpêtrière Hospital, Assistance Publique-Hôpitaux de Paris, Paris, France.

Case report: The patient is a boy aged 14 years with a severe developmental delay, feeding problems and behavioral disorder. He also presents microcephaly, facial dysmorphism, strabismus and fifth digits with bilateral hypoplastic distal phalanges. Using Exome sequencing a heterozygous *de novo* missense variant in *HNRNPR*, p.Arg588His was identified.

Literature: Only five individuals carrying *de novo* variants in *HNRNPR* gene are reported to date (F.A. Duijkers *et al.* 2019*)*. All patients share a similar phenotype to our patient, with developmental delay, hypotonia, feeding problems, behavioral disorder, microcephaly, strabismus, digits abnormalities and facial dysmorphism. Additional symptoms as epilepsy also reported. *HNRNPR* code the heterogeneous nuclear ribonucleoprotein R, involved in RNA expression of human development homeobox and T-box genes. All the reported patients present truncating variants of *HNRNPR*, except one with the same heterozygous missense variant p.Arg588His as our patient.

Discussion: The aim of this work is to report a patient with a *HNRNPR* variant and to establish the pathogenicity of this *de novo* missense *HNRNPR* variant p.Arg588His. Less than 10 patients are reported in literature to date, we thus collect patients for collaborative clinical research on *HNRNPR* variants in order to understand the phenotypic spectrum of this disease.

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P11.062.D A novel frameshift variant in PIEZO1 responsible for hydrops fetalis in a Cypriot family

Andri Miltiadous[1], *Petroula Gerasimou[1], Ioannis Kyprianou[1], Jianxiang Chi[2], Carolina Sismani[3], Paola Evangelidou[3], Angelos Alexandrou[3], Violetta Christophidou Anastasiadou[4], Paul Costeas[1]*

[1]Molecular Hematology-oncology, Karaiskakio Foundation, Nicosia, Cyprus, [2]The Center for the Study of Haematological Malignancies, Nicosia, Cyprus, [3]Cytogenetics Department, Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus, [4]Department of Clinical Genetics, Archbishop Makarios III Medical Centre, Nicosia, Cyprus.

Introduction: Hydrops fetalis is a life threatening condition characterised by accumulation of fluid in a fetus' or a newborn's body compartments. The most common type is non-immune hydrops fetalis (NIHF) which has various causes including cardiovascular and haematological defects and chromosomal aberrations. PIEZO1, a mechanosensitive ion channel has been linked to NIHF cases in an autosomal dominant and recessive manner.

Materials and Methods: Family with three recurrent stillbirths and a newborn that deceased a few days after birth. DNA was extracted from skin tissue of one stillbirth and peripheral blood of the newborn, both presenting with hydrops. Exome sequencing was performed with SureSelect Clinical Research Exome and data analysed with SureCall (Agilent) and ClinicalVarsome (Saphetor). Sanger sequencing confirmed findings. Parents and their first degree relatives were tested by Sanger sequencing.

Results: A PIEZO1 intron-exon junction pathogenic variant, (c.4496-3_4499dupCAGGCCG) was found in homozygous state in the two probands with hydrops fetalis and in heterozygous state in their parents. The variant was inherited from the maternal grandfather, while it was not possible to determine its origin from the paternal side (grandfather deceased). All three maternal aunts did not give any hydrops fetalis births and did not carry this variant.

Conclusions: The PIEZO1 variant presents as an important candidate for the cause of the hydrops fetalis in an autosomal recessive manner in this Cypriot family. Functional studies on RNA level will shed light on the effect of this variant on splicing.

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P11.063.A Role of imprinting disorders in short children born SGA and Silver-Russell syndrome spectrum

Masayo Kagami[1], *Tomoko Fuke[1], Akie Nakamura[1,2], Takanobu Inoue[1], Sayaka Kawashima[1], Kaori Isno-Hara[1], Shinichiro Sano[1,3], Kazuki Yamazawa[1,4], Maki Fukami[1], Tsutomu Ogata[1,3]*

[1]National Research Institute for Child Health and Development, Tokyo, Japan, [2]Hokkaido University Graduate School of Medicine, Sapporo, Japan, [3]Hamamatsu University School of Medicine, Hamamatsu, Japan, [4]National Hospital Organization Tokyo Medical Center, Tokyo, Japan.

Background: (Epi)genetic disorders associated with small-for-gestational-age with short stature (SGA-SS) include imprinting disturbance (IDs). Silver-Russell syndrome (SRS) is a representative ID in SGA-SS and has heterogenous (epi)genetic causes.

Subjects and Methods: To clarify the contribution of IDs to SGA-SS and the molecular and phenotypic spectrum of SRS, we recruited 249 patients with SGA-SS consisting of 92 and 157 patients without structural abnormalities referred to us for genetic testing for SGA-SS and SRS, respectively. 249 patients were classified into three subgroups based on the Netchine-Harbison clinical scoring system (NH-CSS), diagnostic criteria for SRS. We screened various IDs by methylation analysis for differentially methylated regions related to known IDs. We also performed clinical analysis.

Results: These 249 patients with SGA-SS were classified into the SRS-compatible group (n = 148), non-SRS with normocephaly or relative macrocephaly at birth group (n = 94), and non-SRS with relative microcephaly at birth group (n = 7) according to NH-CSS. Various IDs were detected in each group (Table).

Conclusion: We clarified the contribution of IDs as (epi)genetic causes of SGA-SS and the molecular and phenotypic spectrum of SRS. Various IDs constitute underlying factors for SGA-SS, including SRS.

	SRS-compatible	Non-SRS	Non-SRS with microcephaly	Total	
	n = 148	n = 94	n = 7	n = 249	
Genetic causes of SRS	45 (30.4%)	13 (13.8%)	0		
*H19*LOM	38	9	0	47	58/249 (23.3%)
UPD(7)mat	7	4	0	11	
Imprinting disorders other than *H19*LOM and UPD(7)mat	21 (14.2%)	7 (7.4%)	1 (14.3%)		
Temple syndrome	8	3		11	29/249 (11.6%)
UPD(20)mat	4	1		5	
UPD(6)mat	1	2		3	
Prader-Willi syndrome	2		1	3	
11p15 maternal duplication	2	1		3	
UPD(16)mat	2			2	
Parthenogenesis	1			1	
UPD(11)mat mosaic	1			1	
Unknown	82 (55.4%)	74 (78.7%)	6 (85.7%)		162/249 (65.1%)

Abbreviations: SRS, Silver-Russell syndrome; *H19*LOM, loss of methylation of the *H19*/*IGF2*:intergenic differentially methylated region; UPD(7)mat, maternal uniparental disomy chromosome 7; UPD(20)mat, maternal uniparental disomy chromosome 20; UPD (6)mat, maternal uniparental disomy chromosome 6; UPD(16)mat, maternal uniparental disomy chromosome 16; UPD(11)mat, maternal uniparental disomy chromosome 11.

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P11.065.C Oxidative stress elicited by a DNA-demethylating teratogen 5-azacytidine in the mammalian placenta can be alleviated by antioxidant pretreatment

*Nikola Sobocan[1,2], Ana Katusic-Bojanac[3,2], Marta Himelreich-Peric[3,2], Nino Sincic[3,2], Jure Krasic[3,2], Ljiljana Serman[3,4], Davor Jezek[5,4], **Floriana Bulic-Jakus**[3,2]*

[1]Department of Gastroenterology, School of Medicine, University Hospital Merkur, Zagreb, Croatia, [2]Center of Excellence in Reproductive and Regenerative Medicine, School of Medicine, Zagreb, Croatia, [3]Department of Medical Biology, University of Zagreb School of Medicine, Zagreb, Croatia, [4]Center of Excellence in Reproductive and Regenerative Medicine, School of Medicine, Zagreb, Croatia, Zagreb, Croatia, [5]Department of Histology and Embryology, University of Zagreb School of Medicine, Zagreb, Croatia.

Introduction: Our experiments showed that the DNA-demethylating epigenetic drug 5-azacytidine (5azaC) is a teratogen causing oxidative stress and intrauterine growth restriction (IUGR), alleviated in rat fetuses by an antioxidant-pretreatment. We hypothesized such effects will be confirmed in the placenta.

Materials and Methods: On day 12 and 13 of gestation, Fisher rat dams received intravenously N-tert- Butyl-α-phenylnitron (PBN) (40 mg/kg) and one hour later intraperitoneally 5azaC (5mg/kg) or only 5azaC. Controls received PBN or were untreated. Placentas were isolated on the gestation day 15 and day 20. Immunohistochemical signals of the Proliferating Cell Nuclear Antigen (PCNA) and markers of oxidative/nitrosative processes (8-oxoDG and nitrotyrosine, respectively) were stereologically quantified by the numerical density (Nv). The apoptotic index was calculated, and DNA-methylation was assessed by pyrosequencing.

Results: Pretreatment with PBN of 5azaC-treated dams during the trophoblast invasion and intense proliferation phases significantly improved the weight of 15- and 20-days old placentas. In 20-days-old placentas, the apoptotic index and Nv of 8-oxoDG and nitrotyrosine were significantly higher in placentas of dams treated by 5azaC than in those pretreated with PBN. Nv for PCNA was significantly lower in all placentas of 5azaC-treated dams than in controls.

Conclusions: In the placenta, we confirmed the association of DNA-demethylating agent's growth restriction with oxidative/nitrosative stress. These experimental results are of importance for understanding the mechanism of intrauterine growth restriction (IUGR) and its prevention by antioxidant activity. Financed by EU project, ERDF, Operational Programme Competitiveness and Cohesion, No. KK.01.1.1.01.0008, Reproductive and Regenerative Medicine - Exploring New Platforms and Potentials.

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P11.066.D Diagnosis and verification of the autosomal dominant form of Kabuki makeup syndrome in a child with congenital heart disease. Clinical case

Iryna Lastivka[1], *Vita Antsupova[2], Ljudmila Brisevac[3], Larysa Sheiko[3], Iana Ushko[2], Volodymyr Davydiuk[4]*

[1]Bukovinian State Medical University, Chernivtsi, Ukraine, [2]Bohomolets National Medical University, Kyiv, Ukraine, [3]Shupyk National Medical Academy of Postgraduate Education, Kyiv, Ukraine, [4]National Pirogov Memorial Medical University, Vinnitsa, Ukraine.

Introduction: Kabuki Makeup Syndrome (KMS) OMIM 147920 is a rare genetic disease characterized by phenotypic traits, mental retardation, and autistic symptoms. KMS is caused by pathogenic mutations in the genes KMT2D, KDM6A. Mutation of the KMT2D gene is inherited by autosomal dominant type; mutation of the KDM6A gene is inherited X-linked dominantly.

Materials and Methods: A clinical case of KMS in a 2-year-old girl. Syndromological, "Face2gene", cytogenetic, molecular genetic, instrumental methods of examination were used.

Results: Girl from the third planned pregnancy; which was on the background of the threat of miscarriage, polyhydramnios, hypothyroidism. The baby was born at 36 weeks of pregnancy weighing 2930 g, length 49 cm, on the Apgar scale 8/8 points. Phenotype: cleft palate, protruding ear shells, arched eyebrows, long oblique eye slits, blue sclera, epicanthus, ectropion of the lower eyelids, wide tip of the nose, hypoplasia of the nails on the V-fingers of the wrists of the hands, congenital, muscular hypotension, delay of stato-kinetic development. Syndromological diagnostics was performed using the diagnostic program "Face2-gene"; suspected KMS; cytogenetic, molecular genetic research is recommended to verify the diagnosis. Karyotype of a child 46,XX. Molecular genetic analysis revealed a pathogenic mutation (c.11884C>T) (p.Gln3962*) in the KMT2D gene, which is associated with an autosomal dominant type of KMS (MedGen UID: 893727).

Conclusions: Diagnosis of KMS is possible by specific phenotypic characteristics. Verification of the diagnosis is based on the results of molecular genetic analysis. The prognosis of this disease depends on the severity of heart disease and intelligence.

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P11.067.A Clinical features of Malaysians with Kabuki syndrome and identification of *KMT2D* and *KDM6A* mutations by exome sequencing

H Y. Leong, *M A. Haniffa, M Y. Chan, S Sivapatham, H B. Chew, L H. Ngu*

Hospital Kuala Lumpur, Kuala Lumpur, Malaysia.

Introduction: Kabuki syndrome 1 and 2 (OMIM #147920 and #300867) characterized by distinctive facies, learning disability and multiple congenital anomalies are caused by pathogenic mutations in the lysine-specific methyltransferase 2D (*KMT2D*) and lysine-specific demethylase 6A (*KDM6A*) genes respectively.

Methods: This is a descriptive cohort study of Malaysians diagnosed with Kabuki syndrome in Hospital Kuala Lumpur. Whole exome sequencing was performed for variant detection and confirmed by Sanger sequencing.

Results: There were seven Malaysians diagnosed with Kabuki syndrome, 57% males. Their ethnicity were Malay (5/7) and Chinese (2/7). All had global developmental delay and learning difficulty. Five patients had attention deficit and/or hyperactivity. Common dysmorphic features in at least 50% of the cohort included long palpebral fissures, arched eyebrows, broad nasal tip, prominent ears and brachydactyly. Other systemic anomalies reported were short stature (5/7), infantile feeding difficulties (3/7), hearing loss (3/7), cleft lip/ palate (2/7), high arched palate (2/7), developmental dysplasia of the hip (2/7), kyphosis/ scoliosis (2/7), pulmonary artery stenosis (1/7) and multicystic kidney (1/7). Six patients had heterozygous pathogenic *KMT2D* variants (five nonsense and one missense variants). One female patient had a nonsense variant in the *KDM6A* gene. Four variants in the *KMT2D* gene (p.Cys1534Ter, p.Leu3542ValfsTer13, p.Gln4412Ter and p.Glu4422Ter) were novel.

Conclusions: Most Malaysians with Kabuki syndrome in our cohort had truncating mutations leading to haploinsufficiency in the *KMT2D* and *KDM2A* protein. This study expanded our knowledge of the clinical and molecular features of Kabuki syndrome in the South East Asian population.

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P11.068.B Two distinct recessive conditions in two Pakistani sisters: molecular diagnosis using targeted gene panels may require subsequent whole exome sequencing in sibs of consanguineous parents

FRANCESCA PELUSO[1], *Stefano G. Caraffi*[1], *Roberta Zuntini*[1], *Gabriele Trimarchi*[1], *Ivan Ivanovski*[1,2], *Veronica Barbieri*[1], *Maria Marinelli*[1], *Alessia Pancaldi*[3], *Nives Melli*[3], *Claudia Cesario*[4], *Emanuele Agolini*[4], *Elena Cellini*[5], *Renzo Guerrini*[5], *Antonio Novelli*[4], *Giancarlo Gargano*[3], *Orsetta Zuffardi*[6], *Livia Garavelli*[1]

[1]*Medical Genetics Unit, Azienda USL, IRCCS, Arcispedale Santa Maria Nuova, Reggio Emilia, Italy,* [2]*Institut für Medizinische Genetik, Universität Zürich, Zürich, Switzerland,* [3]*Neonatal Intensive Care Unit (NICU), Obstetric and Pediatric Department, Azienda USL, IRCCS, Arcispedale Santa Maria Nuova, Reggio Emilia, Italy,* [4]*Laboratory of Medical Genetics, Bambino Gesù Children's Hospital, IRCCS, Roma, Italy,* [5]*Pediatric Neurology, Neurogenetics and Neurobiology Unit and Laboratories, Meyer Children's Hospital, University of Florence, Florence, Italy,* [6]*Unit of Medical Genetics, Department of Molecular Medicine, University of Pavia, Pavia, Italy.*

We describe two sibilings with two different autosomal recessive conditions born to consanguineous parents. The older sister featured the homozygous c.1416+1del variant in *KATNB1* gene, while the younger had the homozygous c.9729delT variant in *FAT1* gene. The diagnosis in the first sister was achieved by a NGS microcephaly genes panel. After birth of the second sibling, due to the different clinical features not consistent with phenotypic heterogeneity of a *KATNB1*-related syndrome in the same family, we performed Trio Whole Exome Sequencing (WES). The clinical picture in the first child corresponds to Autosomal recessive Lissencephaly 6 and microcephaly (MIM 616212). However, the clinical features of the younger sister consist of bilateral anophthalmia, congenital heart defect (CHD), right split foot with 4 toes and 5 metatarsal, second toe polydactyly, right hand preaxial polydactyly and mild bilateral deafness. The constellation of these features is entirely compatible with a very rare *FAT1*-related condition hitherto described in only 5 families, but including previously unreported clinical features, namely split foot, preaxial polydactyly and CHD. We expand the phenotype of this condition, specifically regarding the involvement of the limbs and heart not previously reported. In the case of consanguineous parents, Trio WES should be prioritised with respect to a panel of genes, since parents could be carriers for two or more different autosomal recessive conditions, and in order to fully delineate recurrence risk for prenatal counselling.

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P11.069.C New pathogenic variant in *GPC4* gene in a patient with Keipert syndrome

Katarzyna Wojciechowska[1], *Borys Styka*[2], *Wiktor Wojczakowski*[1], *Anna Lipińska*[3], *Aleksandra Pietrzyk*[3], *Monika Lejman*[2]

[1]*Department of Pediatric Hematology, Oncology and Transplantology, Children's University Hospital, Lublin, Poland,* [2]*Laboratory of Genetic Diagnostics, Medical University of Lublin, Poland, Lublin, Poland,* [3]*MedGen Medical Centre, Warsaw, Poland, Lublin, Poland.*

Background: Keipert syndrome is a rare X-linked disorder caused by pathogenic variants in *GPC4*gene. In literature, there are only a few such cases discussed. Among the most distinctive features of the patients with Keipert syndrome are: craniofacial and digital abnormalities, learning difficulties and sensorineural deafness. Our aim is to discuss the case of a patient with a new hemizygous mutation in *GPC4*gene.

Methods: Molecular analysis showed a new hemizygous mutation p. Ser236Phe in *GPC4*gene. The genetic analysis was performed by whole-exome sequencing. The library was prepared using Agilent Sureselect VI exome Kit and analyzed with a NovaSeq sequencing platform. The presence of the detected variant was confirmed by Sanger sequencing.

Results: Our patient was born of healthy, nonconsanguineous parents at 39 weeks of gestation by cesarian section. His birth weight was 3040g, length- 52 cm, OFC-35 cm. After birth, dysmorphic facial features were diagnosed. The patient also did not have the right testicle in the scrotum. Karyotyping and array CGH testing showed normal balanced male karyotype. The whole-exome sequencing showed mutation in *GPC4*gene. Among the main health problems of our patient at the age of two years were: short stature (<3rdcentile), delayed motor development (he started sitting without support at the age of 12 months, walking at the age of 24 months) and distinctive dysmorphic facial features.

Conclusion: The case of our patient contributes to the studies of the phenotypes of patients with Keipert syndrome which occur as a result of *GPC4*mutation.

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P11.070.D Mosaicism for a lethal *GJB2* mutation causes Keratitis-Ichthyosis-Deafness (KID) syndrome

Aurora Sánchez Díaz[1,2], *Paula Aguilera*[3,2], *Marta Gracia*[1], *Concha Vidales*[4], *Celia Badenas*[1,2]

[1]*Servicio de Bioquímica y Genética Molecular Hospital Clínic, Barcelona, Spain,* [2]*CIBER de enfermedades raras (CIBERER), Barcelona, Spain,* [3]*Servicio de Dermatología Hospital Clínic, Barcelona, Spain,* [4]*DNA data Biomedical Company, San Sebastian, Spain.*

Keratitis-ichthyosis-deafness (KID) syndrome is a rare congenital disorder caused by autosomal dominant gain of function mutations in the *GJB2* gene that encodes connexin 26 (Cx26). Affected individuals typically are born with erythrokeratoderma and may develop degrees of sensorineural hearing loss and/or progressive vascularizing keratitis. Loss of function *GJB2* mutations are related with nonsyndromic deafness. Some *GJB2* mutations are related with lethal course of KID syndrome by abnormalities in organs other than skin, cornea or inner ear which may contribute to infant death more than severe skin infections. We present a clinically affected KID syndrome patient who is carrier of the G45E mutation in *GJB2* gene in mosaicism. The patient requested the study, previous to undergo artificial reproductive techniques with preimplantation genetic test.

Material and Methods: Sanger sequencing of *GJB2*, NGS panel for erythrokeratodermia and clinical exome were performed.

Results: *GJB2* Sanger study did not detect any mutation. We performed an erythrokeratodermia panel and then a clinical exome. Mutation G45E in *GJB2* gene was detected in 31.25% of reads. Sanger sequencing confirmed the mosaicism for the mutation.

Conclusion: This is the first case affected by KID syndrome due to G45E *GJB2* mosaicism mutation. Heterozygous carriers of G45E *GJB2* mutation, suffer a severe disease and die in infancy. We speculate that only in the mosaic form it is possible the survival of heterozygous carriers. This information is decisive for genetic counseling since the risks for their offspring are extremely low, due to the low probability that germ-line cells carry the mutation.

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P11.071.A Expanding the KIF4A-associated phenotype

Silvia Kalantari[1,2], *Norah Alsaleh*[3], *Ghada M. H. Abdel-Salam*[4], *Fowzan Alkuraya*[5], *Mitsuhiro Kato*[6], *Naomichi Matsumoto*[7], *Satoko Miyatake*[8], *Lucas Fares-Taie*[9], *Caleb Bupp*[10], *Lia Zitano*[10], *Marcello Niceta*[11], *Claudia Cesario*[12], *Roberta Milone*[13], *Bill Dobyns*[14,15,16], *Isabel Filges*[2,17,18]

[1]*Immunogenetics and Transplant Biology Service, Città della Salute e della Scienza University Hospital, Turin, Italy,* [2]*Medical Genetics, Institute of Medical Genetics and Pathology, University Hospital Basel, Basel, Switzerland,* [3]*Division of Medical Genetics and Metabolic Medicine, Department of Pediatrics, Prince Sultan Military Medical City, Riyadh, Saudi Arabia,* [4]*Department of Clinical Genetics, Human Genetics and Genome Research Division, National Research Centre, Cairo, Egypt,* [5]*I4Department of Genetics, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia,* [6]*Department of Pediatrics, Showa University School of Medicine, Tokyo, Japan,* [7]*6Department of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama, Japan,* [8]*Department of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama, Japan,* [9]*7INSERM UMR1163, Imagine – Institute of Genetic Diseases, Paris Descartes University, Paris, France,* [10]*Spectrum Health, Grand Rapids, MI, USA,* [11]*Genetics and Rare Diseases Research Division, Ospedale Pediatrico Bambino Gesù, Rome, Italy,* [12]*Laboratory of Medical Genetics, Ospedale Pediatrico Bambino Gesù, IRCCS, Rome, Italy,* [13]*I11Department of Developmental Neuroscience, IRCCS Fondazione Stella Maris, Calambrone, Italy,* [14]*Center for Integrative Brain Research, Seattle Children's Research Institute, Seattle, WA, USA,* [15]*Department of Pediatrics (Genetics), University of Washington, Seattle, WA, USA,* [16]*Department of Neurology, University of Washington, Seattle, WA, USA,* [17]*Department of Clinical Research, University Hospital Basel, Basel, Switzerland,* [18]*University of Basel, Basel, Switzerland.*

Introduction: KIF4A belongs to the family of kinesins which are molecular motors involved in intracellular trafficking, whose function is critical during human development. KIF4 is involved in the anterograde transport of non-synaptic membrane organelles in developing neurons including the transport of L1 glycoprotein-containing vesicles, being part of L1CAM (L1)-pathway. A disruptive variant in *KIF4A* has been described to cause X-linked intellectual disability and epilepsy. *KIF4A* was also proposed as a candidate gene for congenital hydrocephalus. Our aim is to describe the clinical spectrum associated with *KIF4A* variants.

Materials and Methods: Through GeneMatcher we collected clinical data of 13 patients harboring likely causal variants in *KIF4A*. We compared phenotypes and genotypes in this case series.

Results: We identified 6 patients presenting with congenital hydrocephalus and/or ventriculomegaly with or without associated brain anomalies. 7 Patients had intellectual disability without evidence for brain anomalies. Associated CAKUT, ocular, skeletal and dental anomalies were variably observed. Except for one splice site variant, all other were hemizygous missense variants affecting the kinesin motor or PRC1 binding domain of KIF4A.

Conclusion: This case series allows to extend the phenotypic spectrum associated with *KIF4A* in broadly two categories. Although the number of patients is small, we provide evidence for the role of KIF4A in congenital hydrocephalus and brain anomalies. The functional relationship between L1CAM and KIF4A supports this evidence, but the mechanism needs to be further elucidated. Genotype-phenotype comparison between the group of patients with intellectual disability versus structural brain anomalies so far remains inconclusive.

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P11.072.B Implementation and comprehensive management of a colletion of fetal brain samples obtained from volunteer termination of pregnancies

Purificacion Marin Reina[1], *Inmaculada Lara Cantón*[2], *Álvaro Solaz García*[2], *M Isabel Torres Cuevas*[2], *Iván Millán Yañez*[2], *Jaime Ferrer Lozano*[3], *Vicente Herranz Pérez*[4], *Mª José Ulloa Navas*[4], *Susana Gonzalez Granero*[4], *Francisco Martínez Castellanos*[5], *Ana Gimeno Navarro*[6], *Juan Rubio Moll*[7], *Rosa Gómez Portero*[7], *Ramiro Quiroga de la Cruz*[8], *Raquel Amigo Moreno*[9], *Jose María García Verdugo*[4], *Maximo Vento Torres*[10]

[1]*Hospital UyP La Fe, Valencia, Spain,* [2]*Instituto de Investigación Sanitaria La Fe, Valencia, Spain,* [3]*Pathology Department. Hospital La Fe, Valencia, Spain,* [4]*Cavanilles Institute. University of Valencia, Valencia, Spain,* [5]*Genetics Department. Hospital UyP La Fe, Valencia, Spain,* [6]*Neonatology Department. Hospital UyP La Fe, Valencia, Spain,* [7]*Prenatal Diagnosis. Obstetric Department. Hospital UyP La Fe, Valencia, Spain,* [8]*Prenatal Diagnosis. Obstetric Department. Hospital UyP La FeHospital UyP La Fe, Valencia, Spain,* [9]*Biobank. Hospital UyP La Fe, Valencia, Spain,* [10]*Perinatology Research Unit. Instituto de investigación Sanitaria La Fe., Valencia, Spain.*

Introduction: Obtaining fetal brain samples requires a coordinated multidisciplinary approach. Therefore, a comprehensive management within the framework of an authorized and certified biobank is crucial.

Materials and methods: Prospective and observational study started in September 2019. It included fetuses from legal termination of pregnancies (TOP) from 12 weeks of gestation onwards, with prenatal diagnosis of genetic abnormalities and major malformations. The candidates were identified by the obstetricians, and families were recruited by neonatologists before delivery. After the expulsion, fetuses were either immediately autopsied or preserved overnight at 4ºC and transferred to the Pathology Department within maximum 12 hours. An *in toto* extraction of the brain was made in each case. The right hemisphere was sliced and samples were preserved in 4% paraformaldehyde for ultrastructural studies or snap-frozen at -80ºC, for omic studies, immunohistochemistry analysis or preserved for future research. The left hemisphere was submitted for pathology.

Results: We have recruited 18 fetuses (55,5% affected from aneuploidies) in the first year. Participation in the study was offered to 21 families, of which 19 accepted. We lost 3 cases due to technical problems.In all but one case, the quality of fetal brain tissue was good, as assessed in the gross dissection and in later histologic examination.

Conclusion: 1. Setting up a collection of fetal brain is a complex process which requires a coordinated and motivated team. 2. The biobank facilitates the management of such a collection making it technically and ethically suitable for research.

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P11.073.C Two novel presentations of KCNMA1-Related Pathology - Expanding the Clinical Phenotype of a Rare Channelopathy

Jotte Rodrigues Bento[1], *Candice Feben*[2], *Marlies Kempers*[3], *Maartje Van Rij*[3,4], *Mallory Woiski*[4], *Koenraad Devriendt*[5], *Luc De Catte*[6], *Marcella Baldewijns*[6], *Josephina Meester*[1], *Aline Verstraeten*[1], *Willy Hendson*[7], *Bart Loeys*[1,3]

[1]*Centre of Medical Genetics, Antwerp University Hospital/University of Antwerp, Antwerp, Belgium,* [2]*Division of Human Genetics, National Health Laboratory Service & The School of Pathology, University of the Witwatersrand, Johannesburg, South Africa,* [3]*Department of Human Genetics, Radboud University Medical Center, Nijmegen, Netherlands,* [4]*Department of Gynaecology and Obstetrics, Radboud University Medical Center, Nijmegen, Netherlands,* [5]*Department of Human Genetics, Catholic University of Leuven, Leuven, Belgium,* [6]*Department of Gynaecology and Obstetrics, Catholic University of Leuven, Leuven, Belgium,* [7]*Department of Paediatrics, Rahima Moosa Mother and Child Hospital & The University of the Witwatersrand, Johannesburg, South Africa.*

Background: *KCNMA1* mutations have recently been associated with a wide range of dysmorphological, gastro-intestinal, cardiovascular and neurological manifestations.

Methods: Whole exome sequencing was performed in order to identify the underlying pathogenic mutation in two cases presenting with diverse phenotypical manifestations that did not fit into well-known clinical entities.

Results: In an 8-years old boy presenting with severe aortic dilatation, facial dysmorphism and overgrowth at birth a *de novo* p.Gly375Arg *KCNMA1* mutation, which has been reported previously in association with gingival hypertrophy, aortic dilatation and developmental delay, was identified. Secondly in a 30-week old fetus with severe growth retardation and duodenal atresia a *de novo* p.Pro805Leu *KCNMA1* mutation was identified. The latter has also been reported before in a boy with severe neurological manifestations, including speech delay, developmental delay and cerebellar dysfunction.

Conclusion: The current report presents the first antenatal presentation of pathogenic *KCNMA1* mutation and confirms the specific association of the p.Gly375Arg variant with early onset aortic root dilatation, gingival hypertrophy and neonatal overgrowth.

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P11.075.A An atypical phenotype in a patient carrying a deletion and a missense variant of *LTPB3:* search for overlapping cases for further delineation of the disease

Mathys Wéber[1], *Anne-Sophie Denommé-Pichon*[2,3], *Antonio Vitobello*[2,3], *Patrick Callier*[4,3], *Nadine Hanna*[5], *Pauline Arnaud*[5], *Carine Le Goff*[6], *Pauline Marzin*[7,8], *Catherine Boileau*[5,6], *Agnès Bloch-Zupan*[9,10], *Valérie Cormier-Daire*[7,8], *Christel Thauvin-Robinet*[11,3], *Laurence Faivre*[1,3]

[1]*Centre de Référence Anomalies du Développement et Syndromes Malformatifs, FHU TRANSLAD, Hôpital d'Enfants, CHU Dijon, Dijon, France,* [2]*Unité Fonctionnelle d'Innovation en diagnostic des maladies rares (UF6254), FHU-TRANSLAD, CHU Dijon, Dijon, France,* [3]*Inserm UMR1231 GAD, Génétique des Anomalies du Développement, Université de Bourgogne, Dijon, France,* [4]*Laboratoire de Cytogénétique, Plateau Technique de Biologie, CHU Dijon, Dijon, France,* [5]*Centre National de Référence pour le syndrome de Marfan et apparentés, Hôpital Bichat, APHP, Paris, France,* [6]*Inserm U1148 Laboratoire de recherche vasculaire translationelle, Maladies structurelles cardiovasculaires, Hôpital Bichat, APHP, Paris, France,* [7]*Centre de Référence Maladies Rares MOC, Hôpital Necker-Enfants Malades, APHP, Paris, France,* [8]*INSERM U1163 Institut Imagine, Bases moléculaires et physiopathologiques des chondrodysplasies, IMAGINE - Institut des Maladies Génétiques, Paris, France,* [9]*Centre National pour les Manifestations Bucco-Dentaires des Maladies Rares, ERN CRANIO, Hôpital Civil, CHU de Strasbourg, Strasbourg, France,* [10]*INSERM U1258, CNRS-UMR7104, Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Université de Strasbourg, Illkirch-Graffenstaden, France,* [11]*Centre de Référence Déficiences Intellectuelles de Causes Rares, FHU TRANSLAD, Hôpital d'Enfants, CHU Dijon, Dijon, France.*

Background: *LTBP3*, involved in the TGF-beta signaling pathway, is known to be associated with two autosomal dominant diseases (Geleophysic Dysplasia (GD) and Acromicric Dysplasia (AD)) and an autosomal recessive disease (Dental Anomalies and Short Stature (DASS)). Here we report a patient with an atypical DASS phenotype.

Material and methods: The patient is an 18-year-old male referred for developmental disorders. Since birth, nail and toe brachydactyly with marked hypoplasia of the 3rd and 4th rays and hallux were noted. At tooth eruption stage, microdontia, dyschromia, agenesis and amelogenesis imperfecta appeared. Clinically at age 17, he had short stature (160 cm; -2.5 SD) and a triangular face with severe maxillary prognathism. He also developed ascending aorta dilatation, white matter anomalies, dilatated Virchow-Robin spaces and syringomyelia. Besides, his mother had toe amputations compatible with amniotic bands, dentine defects, syringomyelia and Arnold-Chiari malformation.

Results: Genetic investigations identified two compound heterozygous variants in *LTBP3*. Array CGH first uncovered a maternal inherited heterozygous 3′ partial deletion that could not alone explain the patient's phenotype as his asymptomatic sister also carried it. Targeted re-evaluation of exome sequencing enabled the discovery of a heterozygous missense variant inherited from the father, chr11:g.65308661G>C. This result correlated with the published literature can explain some of the patient's phenotype. However, cerebral, spinal and lower limb abnormalities haven't been described in DASS.

Conclusion: We wondered whether these manifestations could broaden the *LTBP3* phenotype spectrum especially for heterozygous patients. Description of further patients with similar findings would be needed to draw any inferences.

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P11.076.B Luscan-Lumish Syndrome, a fatal disease

Susana García-Linares[1], *Antonio Miguel Poyatos-Andújar*[1], *Margarita Martínez-Atienza*[2], *Irene Sofía Machado-Casas*[1], *Antonio Emilio Jerez-Calero*[1], *Susana Pedrinaci-Rodríguez*[2], *María Luz Bellido-Díaz*[2], *Matías Pérez-Sánchez*[2]

[1]*Hospital Universitario Clínico San Cecilio, Granada, Spain,* [2]*Hospital Universitario Virgen de las Nieves, Granada, Spain.*

Introduction: Luscan-Lumish syndrome is a rare disorder characterized by macrocephaly, intellectual disability, speech delay, low sociability and behavioral problems. More variable features include postnatal overgrowth, obesity, advanced carpal ossification, developmental delay, and seizures. The recurrent c.5218C>T p.(Arg1740Trp) variant in the SETD2 gene appears to be associated with a severe neurodevelopmental disorder with multiple congenital anomalies involving several organ systems. We describe a patient with this variant and fatal outcome (she died from complications of cardiovascular surgery).

Material and Methods: We present a 7-months-old girl who was born after a complicated pregnancy at 37 weeks of gestation (screening of first trimester of high risk and anormal Doppler, aberrant subclavian artery and intrauterine growth restriction in ecography). The patient present at birth absence of suction reflex, hypotonia, perimembranous VSD with increased pulmonary pressures, severe retrognathia and cleft palate. All molecular studies performed during the fetal period were normal. We performed a clinical exome sequencing (CES) analysis at birth.

Results: We found the pathogenic variant c.5218C>T p.(Arg1740Trp) in the SETD2 gene, this variant was determined as *de novo* with the segregation analysis.

Conclusions: Has been described phenotypic heterogeneity associated with SETD2. Our patient have a variant affecting codon 1740 of SETD2, in this case the variant appears to be associated with a severe multisystemic disorder with multiple congenital anomalies involving several organ systems, especially heart, with a fatal outcome.

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P11.077.C Biallelic truncating variants in *MAPKAPK5* cause a new developmental disorder involving neurological, cardiac, and facial anomalies combined with synpolydactyly

Denise Horn[1], *Elisa Fernández-Núñez*[2], *Ricardo Gomez-Carmona*[2], *Ana Rivera-Barahona*[2,3], *Julian Nevado*[3,4,5], *Sarina Schwartzmann*[1], *Nadja Ehmke*[1], *Pablo Lapunzina*[3,4,5], *Ghada A. Otaify*[6], *Samia Temtamy*[6], *Mona Aglan*[6], *Felix Boschann*[1], *Victor L. Ruiz-Perez*[2,4,5,3]

[1]*Institute of Medical Genetics and Human Genetics, Charité – Universitätsmedizin Berlin, Berlin, Germany,* [2]*Instituto de Investigaciones Biomédicas "Alberto Sols", Consejo Superior de Investigaciones Científicas (CSIC)-Universidad Autónoma de Madrid (UAM), Madrid, Spain,* [3]*CIBER de Enfermedades Raras (CIBERER), Instituto de Salud Carlos III (ISCIII), Madrid, Spain,* [4]*ITHACA, European Reference Network on Rare Congenital Malformations and Rare Intellectual Disability, Paris, France,* [5]*Instituto de Genética Médica y Molecular*

(INGEMM)-IdiPAZ, Hospital Universitario La Paz, Universidad Autónoma, Madrid, Spain, [6]Department of Clinical Genetics, Division of Human Genetics and Genome Research, Center of Excellence for Human Genetics, National Research Centre, Cairo, Egypt.

The mitogen-activated protein kinase (MAPK) signaling pathways are involved in key physiological processes such as cell proliferation, differentiation, apoptosis, survival, gene expression, and cell motility. Using exome sequencing, we detected biallelic truncating variants in *MAPKAPK5* in three individuals from two unrelated consanguineous families with a novel syndromic form of neurocardiofaciodigital anomalies. We identified the homozygous frameshift variant c.207_208dupTG; p.(Ala70Valfs*7) in the two affected members of family 1 and the homozygous 1-bp duplication c.1077dup, p.(Leu360Serfs*21) in the patient of family 2. The affected individuals showed a clinically recognizable phenotype characterized by severe developmental delay, variable brain anomalies, congenital heart defects, dysmorphic facial features, and a distinctive type of synpolydactyly. Features also included ophthalmologic abnormalities, hearing impairment, and EEG anomalies. No expression of MAPKAPK5 protein isoforms and reduced levels of the MAPKAPK5-interacting protein ERK3 were detectable in patient derived cells. Furthermore, F-actin recovery after latrunculin B treatment was impaired, indicating that MAPKAPK5 is implicated in F-actin polymerization. Together, our findings show that biallelic loss-of-function variants in *MAPKAPK5* result in a severe developmental disorder and demonstrate a key role of this gene in human brain, heart, and limb development.

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P11.078.D Variable expressivity of 22q11.2 microduplications: an investigation of 13 cases toward a phenotype-genotype correlation

Eunice Matoso[1,2,3], *Alexandra Estevinho*[2], *Susana I. Ferreira*[4], *Luís M. Pires*[4], *Joana B. Melo*[3,4,5], *Fabiana Ramos*[6], *Lina Ramos*[6], *Jorge M. Saraiva*[1,6], *Isabel M. Carreira*[3,4,5]

[1]University of Coimbra, Faculty of Medicine, Coimbra, Portugal, [2]Cytogenetics Laboratory, Medical Genetics Unit, Pediatric Hospital, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal, [3]iCBR- CIMAGO/CACC – Center of Investigation on Environment Genetics and Oncobiology of iCBR/ Clinical Academic Center of Coimbra, Faculty of Medicine, University of Coimbra, Coimbra, Portugal, [4]Cytogenetics and Genomics Laboratory, Faculty of Medicine, University of Coimbra, Coimbra, Portugal, [5]CIBB- Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal, [6]Medical Genetics Unit, Pediatric Hospital, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal.

The 22q11.2 microduplication syndrome shows highly variable phenotypes with reduced penetrance. This region harbors eight segmental duplicated genomic regions termed low-copy repeats A-H (LCR22A-LCR22H) that mediate nonallelic homologous recombination resulting in rearrangements of 22q11.2. The most frequently reported are imbalances with the breakpoints encompassing LCR22A to LCR22D. Atypical deletions and duplications are rare and can provide valuable information of dosage effects of a subset of genes within the 22q11.2 genomic disorder region.We report the identification by array-CGH of 13 patients with microduplications within the 22q11.2 region. Ten of the patients were male and only three female, the clinical phenotypes range from psicomotor delay, microcephaly, dysmorphisms, seizures, skills of behavioral disturbance and cardiopathy, among the most frequently observed. Eight of the patients carry the most common duplication extending between LCR22A-LCR22D, one of them the duplication between LCR22A-LCR22B, two of the patients the atypical duplication between LCR22B-LCR22D and other two the duplication between LCR22D-LCR22E. When available parents were studied, a maternal origin was observed in four patients and paternal origin in one. Attending the pnenotypic variability of the patients, even with similar genomic regions involved, a better clinical characterization is important particularly in duplications sharing a smaller subset of genes. In the more atypical 22q11.2 duplications, like the ones involving LCR22B-LCR22D or LCR22D-LCR22E, a detailed characterization can expand and provide valuable information in the phenotype-genotype interpretation and investigate candidate genes that may be relevant to distinct clinical features.

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P11.079.A MID1 gene variations in a Brazilian cohort with cleft lip with or without palate and ocular hypertelorism. Implication for diagnosis

Roseli M. Zechi-Ceide[1], *Nancy M. Kokitsu-Nakata*[1], *Camila Wenceslau Alvarez*[1], *Chiara Migliore*[2], *Marina Bigeli Rafacho*[1], *Maria Eugênia Siemann*[1], *Rosana M. Candido-Souza*[1], *Siulan Vendramini-Pittoli*[1], *Cristiano Tonello*[3], *Luciana P. Maximino*[4], *Germana Meroni*[5], *Maria Leine Guion-Almeida*[6], *John M. Opitz*[7], *Antonio Antonio Richieri-Costa1*[1]

[1]Department of Clinical Genetics and Molecular Biology, Hospital for Rehabilitation of Craniofacial Anomalies (HRCA), University of São Paulo, Bauru, SP, Brazil., Bauru, Brazil, [2]Department of Life Sciences, University of Trieste, Trieste, Italy, Trieste, Italy, [3]Craniofacial Team, Hospital for Rehabilitation of Craniofacial Anomalies (HRCA, University of São Paulo, Bauru, Brazil, Bauru, Brazil, [4]Department of Audiology and Speech-Language Pathology, School of Dentistry, University of São Paulo, São Paulo, Brazil, Bauru, Brazil, [5]Department of Life Sciences, University of Trieste, Trieste, Italy., Trieste, Italy, [6]Hospital for Rehabilitation of Craniofacial Anomalies, University of São Paulo (HRCA-USP)., Bauru, Brazil, [7]Division of Medical Genetics, Department of Pediatrics, Human Genetics, Pathology, and Obstetrics & Gynecology, University of Utah, Salt Lake City, Utah, USA., Salt Lake City, UT, USA.

Introduction: Orofacial clefts is the most common craniofacial anomalies. It occur isolated or associated to other anomalies. The combination of cleft lip and ocular hypertelorism can represents the Opitz G/BBB syndrome, a rare genetic condition with hypospadia, laryngo-tracheo-esophageal abnormalities, and heart and brain defect. The X-linked form is caused by MID1 gene.

Methods: Screening of the MID1 gene variation (Sanger Sequencing) or deletion (MLPA) was performed in 44 patients with cleft lip with or without cleft palate and ocular hypertelorism, enrolled in HRCA-USP. All patients and their families were evaluated by a craniofacial clinical geneticist.

Results: MID1 gene variations were found in 22 individuals (21 male and 1 female). Four missense variations were not previously reported and were considered of unknown significance. The heterozygous variations c.1305A>G was found in an affected girl. About 95% of the patients had a bilateral cleft lip and palate. Other frequent clinical signs were hypospadia (~85 %), brain anomalies (100% of 11 images evaluated) and learning difficulties (42%). Dandy-Walker malformation was present in 72% of the 11 images evaluated.

Conclusion: MID1 gene contributes with occurrence of cleft lip associated to ocular hypertelorism and this combination can be a mild form of the G/BBB syndrome. The expanding of frontonasal process can be contributed with the fusion of upper lip and palate failure. Despite of Dandy-Walker malformation is present in 100% of the images evaluated, the development was normal in most patients and learning difficulties was a frequent feature.

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P11.080.B *MNS1* gene alterations are associated with *situs inversus* and male infertility

Joseph Leslie[1], *Lettie E. Rawlins*[1,2], *Barry A. Chioza*[1], *Oluwaseun R. Olusanya*[1], *Claire G. Salter*[1], *James Fasham*[1], *Harold E. Cross*[3], *Simon Lam*[1], *Gaurav V. Harlalka*[1], *Martina M. A. Muggenthaler*[1,4], *Emma L. Baple*[1,2], *Andrew H. Crosby*[1]

[1]*Institute of Biomedical and Clinical Science, RILD Wellcome Wolfson Centre, University of Exeter Medical School, Exeter, United Kingdom,* [2]*Peninsula Clinical Genetics Service, Royal Devon & Exeter Hospital, Exeter, United Kingdom,* [3]*Department of Ophthalmology, University of Arizona College of Medicine, Tucson, AZ, USA,* [4]*Department of Cardiology, Royal Devon and Exeter NHS Foundation Trust, Exeter, United Kingdom.*

Motile cilia are hair like structures on the cell surface that protrude into the extracellular space and beat in a coordinated manner. This performs a number of important roles in the body including the clearance of mucus, the propulsion of sperm and the determination of left-right patterning during development. Ciliopathy disorders arise due to abnormalities in the function of motile cilia and encompass a group of autosomal recessive conditions typified by chronic otosinopulmonary disease, infertility and *situs* abnormalities.

We used a combination of genetic studies to investigate the cause of a ciliopathy disorder identified in four Amish individuals with *situs inversus* and male infertility. Genome-wide SNP mapping identified a single small (2.34Mb) autozygous region common to all four affected individuals of on chromosome 15q21.3, demarcating the likely disease locus. Whole exome sequencing identified a single candidate variant genome wide, located in the autozygous region, in the *MNS1* gene (NM_018365.2: c.407_410del;p.(Glu136Glyfs*16)). Genotyping of members of the extended family identified randomisation of the laterality defects in other affected individuals homozygous for the *MNS1* alteration, while all unaffected individuals were wild type or heterozygous.

Our studies in the Amish are consistent with previous investigations of MNS1 deficient mice, which display *situs* randomisation and male infertility. Importantly, a number of other studies have recently been published identifying *MNS1* mutations in individuals worldwide with situs abnormalities and/or male infertility. Together these findings identify *MNS1* alterations as a potential under recognised cause of male infertility and highlight the importance of including *MNS1* on gene testing panels globally.

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P11.081.C Unraveling the genetic causes of Moebius syndrome

Giada Moresco[1], *Ornella Rondinone*[1], *Jole Costanza*[1], *Carlo Santaniello*[1], *Laura Fontana*[1,2], *Alessia Mauri*[1], *Marco Venturin*[3], *Odoardo Picciolini*[4], *Roberta Villa*[5], *Monica Miozzo*[1,2], *Maria Francesca Bedeschi*[5]

[1]*Research Laboratories Coordination Unit, Fondazione IRCCS Ca Granda Ospedale Maggiore Policlinico, Milano, Italy,* [2]*Department of Health Science, Università degli Studi di Milano, Milano, Italy,* [3]*Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, Milano, Italy,* [4]*Pediatric Physical Medicine and Rehabilitation Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy,* [5]*Medical Genetic Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy.*

Moebius syndrome (MBS; OMIM #157900) is a rare congenital disorder characterized by nonprogressive facial and ocular abduction paralysis, and impairment to the facial (cranial VII) and abducens (cranial VI) nerves, respectively, possibly due to hindbrain defects. Additional features can include hearing loss, other cranial nerve dysfunctions, motor, orofacial, musculoskeletal, neurodevelopmental and behavioral complications. Most are sporadic cases, but familial occurrence has also been reported, with both autosomal dominant/recessive and X-linked inheritance. Diagnostic molecular criteria for MBS are still undefined. *De novo* heterozygous pathogenetic variants of *REV3L* and *PLXND1* genes are considered to be causative for MBS, although they occur in a minority of cases. With the aim to uncover novel genes associated with this condition, we recruited a cohort of 35 Moebius and Moebius-like patients and performed trio-WES. No *de novo* variants were identified in the *REV3L* and *PLXND1* genes, strongly suggesting the need to discover possible additional causative genes. We selected the most frequently mutated genes among the 35 probands, and performed Ingenuity Pathway Analysis (IPA) to identify possibly altered pathways. Our preliminary results suggest that axon guidance pathways, such as the Semaphorin and Netrin signaling pathways, are likely to be involved in the disease pathogenesis, as well as GP6, nNOS and protein kinase A signaling pathways.

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P11.082.D Expanding the phenotype of *NADSYN1* associated congenital NAD deficiency disorder - the first reported adult patient

Emilie Erbs[1], *Claus Lohmann Brasen*[2,3], *Maria Rasmussen*[1,3]

[1]*Department of Clinical Genetics, Lilebælt Hospital, Vejle, Denmark,* [2]*Department of Biochemistry and Immunology Lillebælt Hospital, Vejle, Denmark,* [3]*Department of Regional Health Research, University of Southern Denmark, Odense, Denmark.*

Introduction: Nicotinamide adenine dinucleotide (NAD) is an essential coenzyme in multiple cellular redox processes and a substrate for signalling enzymes in non-redox reactions. *NADSYN1* encodes NAD synthetase 1 an enzyme in the final step of the NAD *de novo* synthesis pathway and part of the Preiss-Handler pathway. Szot et al. recently reported five patients from four families with bi-allelic deleterious variants in *NADSYN1*. These patients all had kidney anomalies and some had additional malformations of the heart, vertebrae and limbs. Neither of the patients had survived past three months postnatally.

Materials and methods: Here we present a 30-year-old male patient with a height of 130 cm and numerous skeletal malformations including segmentation defects of the spine, rib anomalies and unequal leg length. Additionally, the patient had bilateral ptosis, cleft palate and asymmetric dysmorphic facial features. The patient underwent surgery for an aortic stenosis due to a bicuspid valve, but no malformations of the kidneys or urinary tract have been identified. Intelligence was normal.

Results: Trio exome sequencing revealed a variant in *NADSYN1* c.1717G>A (p.Ala573Thr) in homozygous form. Both parents were carriers of the variant in heterozygous form.

Conclusion: This missense variant was reported in three of the patients described by Szot et al. Previous functional analyses have supported the pathogenicity of the variant. We report the first adult patient with *NADSYN1* associated congenital NAD deficiency disorder and thus greatly expand the phenotypic spectrum. Photos of the patient and measurements of NAD metabolites will be provided.

E. Erbs: None. **C. Brasen:** None. **M. Rasmussen:** None.

P11.083.A Functional analysis of a novel 5'UTR variant of the *LMX1B* gene associated with a familial case of Nail-Patella Syndrome

Serena Cappato[1], *Aldamaria Puliti*[1,2], *Maria Teresa Divizia*[2], *Margherita Lerone*[2], *Federico Zara*[1,2], *Renata Bocciardi*[1,2]

[1]*Dept of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Sciences (DINOGMI), University of Genova, Genova, Italy,* [2]*UOC Genetica Medica, IRCCS Giannina Gaslini, Genova, Italy.*

Introduction: Nail-Patella Syndrome (NPS, MIM#161200) is an autosomal dominant disorder due to mutation or partial/complete deletion of the LMX1B gene, causing haploinsufficiency. Dysplasia of the nails, absent/hypoplastic patellae, presence of iliac horns and elbow deformities are the cardinal features. Glaucoma and renal involvement from subclinical hematuria/proteinuria to end stage renal failure can be also present.A family with recurrence of NPS was negative for canonical molecular and cytogenetic analysis of the LMX1B gene. The screening of the 5'UTR of the gene revealed the presence of a novel heterozygous c.-174C>T variant, segregating with the phenotype.

Materials and Methods: Investigation of the role of the variant followed this workflow: in silico analysis of the 5'UTR; study of allele-specific expression in patients derived cells; evaluation of the impact of 5'UTR variant, relative to the WT allele, on LMX1B expression in heterologous cell-based assays.

Results: In postnatal life, expression of LMX1B is restricted to poor accessible tissues. Therefore, we established a protocol to induce its expression in patients and controls derived lymphoblasts and observed that the mutated allele was less expressed at RNA level. The analysis of the WT and 5'UTR variant in heterologous cell-based assays demonstrated a negative effect of the variant on LMX1B protein expression, according to haploinsufficiency mechanisms suggested for NPS.

Conclusions: 5'UTR sequences play an important role in the regulation of gene expression by different mechanisms. We provide evidence that a variant in the 5'UTR of LMX1B can affect expression of the gene and may be involved in NPS pathogenesis.

S. Cappato: None. **A. Puliti:** None. **M.T. Divizia:** None. **M. Lerone:** None. **F. Zara:** None. **R. Bocciardi:** None.

P11.084.B Three Nance Horan syndrome families from Turkey; Three different approaches for molecular diagnosis

Hilal Pırıl P. Saraçoğlu[1], *Yeliz Güven*[2], *Sermin Dice Aksakal*[2], *Tuğba Kalaycı*[3], *Umut Altunoğlu*[4,5], *Zehra Oya Uyguner*[6], *Serpil Eraslan*[5], *Esra Börklü-Yücel*[5], *Hülya Kayserili Karabey*[4,5,7]

[1]*Koç University, Graduate School of Health Science, Cellular and Molecular Medicine, İstanbul, Turkey,* [2]*İstanbul University, Faculty of Dentistry, Department of Pedodonty, İstanbul, Turkey,* [3]*İstanbul University, Faculty of Medicine, Department of Internal Medicine, İstanbul, Turkey,* [4]*Koç University, School of Medicine, Department of Medical Genetics, İstanbul, Turkey,* [5]*Koç University Hospital, Genetic Diseases Evaluation Center, İstanbul, Turkey,* [6]*İstanbul University, Faculty of Medicine, Department of Medical Genetics, İstanbul, Turkey,* [7]*Koç University, Graduate School of Health Sciences, İstanbul, Turkey.*

Nance-Horan Syndrome (NHS,MIM: #302350) is an X-linked dominant disease, featuring ocular abnormalities (congenital cataracts, microphthalmia), dental abnormalities, facial dysmorphisms and intellectual disability and/or autism. Males show full penetrance with variable expressivity while carrier females manifest mild features. It is caused by loss-of-function variants in *NHS* (Xp22.2). A regulator of actin remodeling, NHS may orchestrate actin regulatory protein function in response to signaling events during development. It is ultra-rare, with 60 reported cases till 2018. Patient1(P1) was clinically diagnosed with NHS at 15 years, due to bilateral cataracts and microcornea, Hutchinson incisors, mild intellectual defect. His mother had mild cataracts. Both had heterochromia iridis as additional finding. Their diagnoses were confirmed by sequencing. Patient2(P2), boy aged 6 months, was evaluated due to global developmental delay accompanying microphthalmia, cataracts, VSD. SNP-array testing was performed, with preliminary diagnosis of syndromic microphthalmia, revealing a deletion encompassing *NHS*. In Family3(F3), maternal half-brothers [23(P3), 36(P4)] and their maternal uncle(P5), had congenital cataracts. Additionally, F3 was consulted due to neurobehavioral findings. P3's solo-WES analysis revealed an *NHS* variant, segregating with the phenotype.

Patient	**Methods**	
P1;Mother	Sequencing	c.246C>T (hemizygote;heterozygote)
P2	SNP-Array	2.26Mb deletion
P3	WES	c.1867delC

Here, we report five affected males and a manifesting female from three unrelated families. While direct sequencing confirmed the clinical diagnosis of NHS in P1, P2 not showing distinct features of NHS, algorithmic diagnostic approach was applied. F3 had pedigree suggestive for X-linked inheritance and WES, widely used technique, was utilized.

H.P. Saraçoğlu: None. **Y. Güven:** None. **S. Aksakal:** None. **T. Kalaycı:** None. **U. Altunoğlu:** None. **Z. Uyguner:** None. **S. Eraslan:** None. **E. Börklü-Yücel:** None. **H. Kayserili Karabey:** None.

P11.085.C Genetics of neural tube defects: new candidate genes and complex mode of inheritance

Marie Faoucher[1,2], *Artem Kim*[1], *Marie Beaumont*[1], *Wilfrid Carre*[1,2], *Hubert Journel*[3], *Linda Akloul*[4], *Laurent Pasquier*[4], *Mélanie Fradin*[4], *Chloé Quelin*[4], *Andrea Manunta*[5], *Erwan Watrin*[2], *Marie De Tayrac*[1,2], *Sylvie Odent*[4,2], *Christèle Dubourg*[1,2], *Valérie Dupé*[2], *Véronique David*[1,2]

[1]Genetics laboratory, CHU Pontchaillou, Rennes, France, [2]UMR 6290 CNRS, IGDR, Univ Rennes1, Rennes, France, [3]Genetics department, CH Vannes, Vannes, France, [4]Genetics department, CHU Pontchaillou, Rennes, France, [5]Urology department, CHU Pontchaillou, Rennes, France.

Neural tube defects (NTDs) are developmental disorders that affect approximately 1 out of 1000 pregnancies and result in an incomplete closure of the neural tube. Defects in the planar cell polarity (PCP) and the folates metabolism pathway have been strongly associated with NTDs in animal models and recent studies of human cohorts. Furthermore, multifactorial and oligogenic pattern of inheritance have been suggested for this rare disease. We present high throughput sequencing results from a cohort of 102 patients with NTDs. Medical Exome Sequencing or Whole Exome Sequencing was performed on 74 solo cases and 28 families. Variants analysis was focused on an *in silico* panel of 250 genes previously implicated in NTDs.Deleterious variants in candidate genes have been detected in more than half of the cohort. Most represented pathways in terms of mutational frequencies are: PCP (32% of variants), folate metabolism (15%), embryonic development (11%), *SHH* pathway (8.4%), apoptosis genes (7%) and primary cilia (4.2%). The most recurring genes are *CELSR1* (8 patients), *FREM2* (5), *GLDC* (4) or *APAF1* (3). Moreover, we describe oligogenic mode of inheritance in 33% of our families. These results confirm the main implication of PCP and folate genes and enhance the role of *SHH* pathway, apoptosis and cilium genes in NTDs. Functional explorations are needed to confirm the implication of these genes and oligogenic combination analysis on our cohort is necessary to investigate the complex mode of inheritance of NTDs.

M. Faoucher: None. **A. Kim:** None. **M. Beaumont:** None. **W. Carre:** None. **H. Journel:** None. **L. Akloul:** None. **L. Pasquier:** None. **M. Fradin:** None. **C. Quelin:** None. **A. Manunta:** None. **E. Watrin:** None. **M. De Tayrac:** None. **S. Odent:** None. **C. Dubourg:** None. **V. Dupé:** None. **V. David:** None.

P11.086.D Novel homozygous missense mutation in *NARS1* gene: A new neurodevelopmental disorder with microcephaly

Sehime Gulsun TEMEL[1], *Sebnem Ozemri Sag*[1], *Erdal Eren*[1], *Engin Deniz*[2]

[1]Bursa Uludag University, BURSA, Turkey, [2]Yale University, New Haven, CT, USA.

Aminoacyl-tRNA synthetases (ARSs) are enzymes essential for protein translation. Asparaginyl-tRNA synthetase1 (NARS1) belongs to the class IIa family, based upon a 7 beta-strand protein structure, and functions in the cytoplasm responsible for asparagine tRNA charging in these locations. More recently it was reported that de Novo and Bi-allelic pathogenic variants in NARS1 Cause neurodevelopmental delay due to toxic gain-of-function and partial loss-of-function effects. We report 10 month old deceased boy who initially presented with microcephaly and dismorphic features. He was the second-born from a consanguineous marriage at 33+5 week gestational age. Clinical findings included resistant epilepsy, neurodevelopmental delay, neonatal diabetes, inguinal hernia, hydrocele testis, humoral immunodeficiency. Multiple cavernous malformation were observed on his brain tomography. ECHO findings revealed VSD and pulmonary stenosis. Exome sequencing revealed a homozygous missense mutation c.866A>G, (p.Tyr289Cys) in the *NARS1* gene. The segregation of this rare variant in the family was confirmed by Sanger sequencing. The 3D structure of the mutant protein was predicted computationally. Based on our modeling analyses we can speculate on two different effects of Tyr289Arg mutation. First, mutation of aromatic bulky Tyr to non-aromatic much less bulky Cys amino acid may inhibit the NARS homodimer formation or weaken the interaction between chains of the homodimer. Second, since Tyr289 is in the tRNA and NARS binding region, Tyr289Cys mutation may attenuate the specific tRNA-enzyme binding.

S.G. Temel: None. **S. Ozemri Sag:** None. **E. Eren:** None. **E. Deniz:** None.

P11.087.A Position effect and of modifier Ras pathway genes in Neurofibromatosis type I microdeletion syndrome

Paola Bettinaglio[1,2], *Viviana Tritto*[1], *Eleonora Mangano*[3], *Roberta Bordoni*[3], *Chiara Castronuovo*[3], *Marinella Volontè*[1], *Claudia Cesaretti*[4], *Giulia Anna Cagnoli*[4], *Cristina Battaglia*[1,3], *Veronica Saletti*[5], *Donatella Bianchessi*[6], *Federica Natacci*[4], *Marica Eoli*[6], *Paola Riva*[1]

[1]Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, Segrate, Italy, [2]Università degli Studi di Brescia, Brescia, Italy, [3]Institute of Biomedical Technologies, Consiglio Nazionale delle Ricerche, Segrate, Italy, [4]Medical Genetics Unit, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milano, Italy, [5]Developmental Neurology Unit,Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy, [6]Molecular Neuroncology Unit,Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy.

Introduction: Neurofibromatosis type1(NF1) microdeletion syndrome (MD)accounting for 5-11% of NF1 patients is characterized by a severe phenotype.70% of patients show the 1.4 Mb type1 deletion and variable expressivity of the phenotype suggesting the involvement of different mechanisms.

Materials-And-Methods: We studied 19 NF1-type1-MD patients by targeted-NGS analysis with a panel of RAS/MAPK pathway genes, genes within and flanking the 17q11.2 microdeletion, to investigate pseudo-dominance and genetic modifier effect.We assayed position effect of deleted region by gene expression analysis of 10 genes flanking the deletion by RT-PCR on RNA from peripheral blood comparing a subgroup of 15 NF1-MD patients with 15 patients with an *NF1* gene mutation.Haploinsufficiency was established by evaluating the probability of LoF intolerance.

Results: We identified 14rare variants(Table1) and classified "likely pathogenic"two variants in Ras pathway genes RAF1 and RASA1.The RAF1 variant is present in a patient with a cerebrovascular pathology while the RASA1 in a patient with an uncommon glioma of the brainstem developed during infancy. Expression analysis showed five hypo-expressed(*IFT20*,*SSH2*, *RHOT1*,*ZNF207*,*PSMD11*) and two over-expressed genes(*ABHD15*, *BLMH*) in NF1-MD patients compared to classical NF1 patients,with a statistical significance of $p < 0.05$.Furthermore,we found that ATAD5,NF1,OMG,RAB11FIP4,andSUZ12 genes are intolerant to haploinsufficiency.

Conclusions: Besides haploinsufficiency,position effect of NF1 microdeletion and possible modifier gene variants of Ras pathway could have a role in phenotype severity.Further genetic and functional studies in a larger cohort of NF1-type1-MD will be performed to improve genotype-phenotype correlation.

Table 1. Rare variants(MAFeur1000g < 0.01)identified in *NF1*type1-microdeletion patients

PatientID	Gene	Exon	dbSNP build 154	Nucleotide variation	Protein variation
N22	*A2ML1 NM_001282424.2*	16	rs200964353	c.1796G>A	p. Gly599Asp
N22	*CPD NM_001199775.1*	19	-	c.2929G>A	p. Gly977Arg
N26		5	rs61749868	c.1245G>T	

Table 1. Rare variants(MAFeur1000g < 0.01)identified in *NF1*type1-microdeletion patients

PatientID	Gene	Exon	dbSNP build 154	Nucleotide variation	Protein variation
	RNF135 NM_032322.4				p. Trp415Cys
N27	*GAB2 NM_012296.3*	4	rs561641037	c.862A>T	p. Ile288Phe
N27	*RASAL1 NM_001193521.1*	16	rs142556970	c.1804T>C	p. Phe602Leu
N28	*PHF12 NM_001033561.2*	8	-	c.1246C>G*	p. His416Asp
N43	*RAF1 NM_002880.3*	17	-	c.1811C>G	p. Ser604Cys
N75	*RASAL2 NM_004841.4*	6	-	c.898C>G*	p. Gln300Glu
N75	*RASA1 NM_002890.3*	20	-	c.2656C>T	p. Pro886Ser
N76	*SARM1 NM_015077.4*	9	rs144613221	c.1498T>C	p. Tyr500His
N80	*LRP1 NM_002332.3*	51	rs962402779	c.8218G>A	p. Glu2740Lys
N80	*PAK4 NM_001014834.3*	3	-	c.449A>G*	p. Gln150Arg
N82	*RASAL3 NM_022904.3*	13	-	c.1983G>A*	p. Met661Ile
N83	*GAB2 NM_012296.3*	3	rs770269898	c.350A>G	p. Glu117Gly

*Novel variants, not reported in any of the consulted databases

P. Bettinaglio: None. **V. Tritto:** None. **E. Mangano:** None. **R. Bordoni:** None. **C. Castronuovo:** None. **M. Volontè:** None. **C. Cesaretti:** None. **G. Cagnoli:** None. **C. Battaglia:** None. **V. Saletti:** None. **D. Bianchessi:** None. **F. Natacci:** None. **M. Eoli:** None. **P. Riva:** None.

P11.088.B Whole genome sequencing reveals the breakpoints disrupting the NHS gene on a balanced pericentric inversion in a patient with Nance-Horan syndrome

Alejandra Damián[1,2], *Raluca O Ionescu*[3], *Marta Rodríguez de Alba*[1,2], *Alejandra Tamayo*[1,2], *María José Trujillo*[1,2], *María Carmen Cotarelo*[3], *Olga Pérez*[3], *Lorena de la Fuente*[1,2], *Pablo Mínguez*[1,2], *Cristina Villaverde*[1,2], *Carmen Ayuso*[1,2], *Marta Corton*[1,2]

[1]*Instituto de Investigación Sanitaria Fundación Jiménez Díaz, Madrid, Spain,* [2]*Centre for Biomedical Network Research on Rare Diseases, Madrid, Spain,* [3]*University Hospital Clínico San Carlos, Madrid, Spain.*

Introduction: Inversions are structural genomic variants that are generally balanced and detected by cytogenetic approaches. However, they could lead to gene disruptions or even have positional effects leading to diseases. Nance-Horan syndrome is an X-linked disorder characterized by congenital cataracts, dental anomalies, and other features. It is caused by mutations in the *NHS* gene on chromosome Xp22.13. Here, we present a strategy to characterize the breakpoints by Whole Genome Sequencing (WGS) on an apparently balanced pericentric inversion X (p22.13-q27.3), maternally inherited, in a child with syndromic bilateral cataracts and its implications in the phenotype.

Material and methods: 30X short-read Illumina paired-end WGS was performed in the proband and inversion breakpoints were confirmed by PCR of the specific fragment junctions and Sanger sequencing. EdU incorporation on lymphocytes culture and FISH analysis were used to asses skewed X-inactivation patterns. Expression of involved genes was evaluated by ddPCR-based Taqman analysis from blood RNA.

Results: We characterized the breakpoint position in Xp22.13, with a 15pb deletion, disrupting the intron 1 of *NHS*. In Xq27.3, the breakpoint is situated in an intergenic region. A microhomology of 5 pb (TTATA) was found in both sides. Skewed X-inactivation was not detected in his clinically unaffected mother. Topologically associated domains (TADs) are disrupted by the inversion.

Conclusions: We report the first chromosomal inversion disrupting the *NHS* gene, efficiently characterized by WGS, likely caused by microhomology-mediated end-joining mechanism. Moreover, we discussed the implication of flanking genes and X inactivation on the differential features or expressivity of the phenotype.

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P11.089.C Prenatal detection of a new disease-causing mutation in a preterm neonate with NP-C disease

Sinje Geuer

Bioscientia, Ingelheim, Germany.

Niemann-Pick C disease (NP-C) is an ultra-rare, autosomal recessive, neurovisceral lysosomal lipid storage disorder (LSD) with impaired intracellular lipid trafficking, and an estimated incidence of at least 1/120 000 live births. The first neurological symptoms vary with age of onset and include delay of developmental motor milestones, gait disturbances, increased falls, clumsiness, cataplexy, supranuclear gaze palsy, school problems and ataxia. Other common features are seizures and dystonia as well as dementia. Due to the extreme rarity of NP-C disease, establishing a correct diagnosis is challenging, and is often realized with a substantial delay. Here we report a preterm male with postnatal severe respiratory problems, hepatosplenomegaly, bilateral hydroceles, anemia, hyperbilirubinemia, but no neurological abnormalities. Prenatally, antenatal scans at 25 weeks of gestation demonstrated ascites. Prenatal trio whole exome sequencing (WES) detected two compound-heterozygous frameshift mutations in the gene *NPC1* of which one was novel (c.3294dup p.(Ile1099Tyrfs*22)). This result yielded to the diagnosis of NP-C disease. Thus early diagnosis of rare and ultra-rare metabolic diseases (eg, LSDs) can be facilitated by using (prenatal) trio exome analysis, thus establishing an early definite diagnosis.

S. Geuer: A. Employment (full or part-time); Modest; Bioscientia.

P11.090.D Genetic and phenotypic spectrum in the *NONO*-associated syndromic disorder

Franziska Roessler[1], *Tobias Bartolomaeus*[2], *Norman Delanty*[3], *Marie T Greally*[4], *Chiara Klöckner*[1], *Bernt Popp*[1], *Michael Weidenbach*[5], *Dominik Westphal*[6], *Cordula M Wolf*[7], *Vincent Strehlow*[1]

[1]*University of Leipzig Medical Center, Leipzig, Germany,* [2]*Praxis für Humangenetik, Tübingen, Germany,* [3]*FutureNeuro SFI Research Centre, The Royal College of Surgeons in Ireland, Dublin, Ireland,* [4]*School of Pharmacy and Biomolecular Sciences, The Royal College of Surgeons in Ireland, Dublin, Ireland,* [5]*Department of Pediatric Cardiology, Helios Leipzig Heart Center, Leipzig, Germany,* [6]*Institute*

of Human Genetics, Technical University of Munich, Munich, Germany, [7]Department of Congenital Heart Defects and Pediatric Cardiology, German Heart Center Munich, Technical University Munich, DZHK (German Centre for Cardiovascular Research), partner site Munich Heart Alliance, Munich, Germany.

The non-POU domain-containing octamer-binding (NONO) protein is involved in multiple steps of gene regulation such as RNA metabolism and DNA repair. Hemizygous pathogenic variants in the underlying X-chromosomal *NONO* gene were confirmed to cause a rare syndromic disorder. Through our in-house diagnostics and subsequent matchmaking, we identified three unrelated male individuals with pathogenic *NONO* variants. For detailed comparison, we reviewed all published characterizations of the *NONO*-associated disorder. To date, ten live-born and seven prenatal cases with either maternally inherited or *de novo* variants in *NONO* were reported in fourteen families. All live-born individuals demonstrated global developmental delay. Most had heart malformations (7/10), consistently including non-compaction cardiomyopathy (7/7). Brain abnormalities were reported in 8/10, consistently including dysgenesis or thickening of the corpus callosum (8/8). Seizures were described once in an individual additionally carrying a *de novo* 15q13.3 microdeletion. Prenatal cases were defined by their cardiac phenotype encompassing non-compaction cardiomyopathy (5/7), cardiomegaly (1/7) and hypoplastic left heart syndrome (1/7). Variant spectrum comprised truncating (10/14) and splice (4/14) variants. Our three patients carry two unique frameshifting variants and a novel splice variant. RT-PCR showed that the c.651-1G>C variant causes an in-frame deletion of 32 amino acids (p.Phe218_Lys249del). Modelling indicated that the aberrant protein is likely misfolded and degraded. Based on our cohort and literature review, we provide a comprehensive overview of the genetic and phenotypic spectrum in the *NONO*-associated syndromic disorder. We further undermine loss-of-function as the pathomechanism and extend the phenotypic spectrum through a second case with epilepsy but without heart malformations.

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P11.091.A Molecular and clinical spectrum of patients included in RASopathies group with Noonan phenotype from a Romanian cohort

VASILICA PLAIASU[1], *Diana Ozunu*[1], *Gabriela Motei*[1], *Mihaela Ivan*[1], *Camelia Procopiuc*[2], *Iuliana Gherlan*[2], *Luiza Vitan*[3]

[1]*INSMC Alessandrescu-Rusescu, Regional Center of Medical Genetics, Bucharest, Romania,* [2]*National Institute of Endocrinology CI Parhon, Bucharest, Romania,* [3]*Clinical Hospital CF2, Bucharest, Romania.*

Background: The RASopathies are a group of rare genetic conditions caused by mutations in genes of the Ras-MAPK pathway but together represent one of the most common groups of genetic disorders, affecting approximately 1 in 1,000 individuals. Most individuals with RASopathies share common phenotypes, such as a short stature, heart defects, facial abnormalities, and cognitive impairments, but with vast heterogeneity in clinical and genetic features.

Materials and Methods: We describe 7 unrelated probands with clinical features highly suggestive of Noonan syndrome. Genetic analyses using RASopathy genetic panel comprising 18 genes (PTPN11, BRAF, CBL, HRAS, KAT6B, KRAS, LZTR1, MAP2K1, MAP2K2, NF1, NRAS, RAF1, RASA2, RIT1, SHOC2, SOS1, SOS2, SPRED1) or WES were applied.

Results: We retrospectively reviewed the mutation spectrum and clinical outcome of patients with Noonan phenotype, confirmed by molecular testing. Phenotypic data recorded included all clinical characteristics: abnormal growth, facial, cardiac, thorax, and other features: learning difficulties, ocular anomalies, cryptorchidism, and disorders of pubertal timing, lymphatic anomalies. Our study highlights the role of molecular genetic testing in the process of differential diagnosis of Noonan syndrome. Among these mutations, the majority were previously reported and predicted to be pathogenic: PTPN11-c.923A>G for two cases, SHOC2-c.4A>G, RAF1-c.788T>C, KRAS-c.178G>C, except one of SHOC2 gene with paternal inheritance c.1582C>T which is unknown.

Conclusions: Early, accurate diagnosis of Noonan syndrome has important implications for genetic counseling and monitoring for a large number of potential health conditions. The clinical profile and the genetic heterogeneity of RASopathies could make choosing between panel testing and WES analysis difficult.

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P11.092.B European Medical Education Initiative on Noonan Syndrome: A clinical practice survey assessing the diagnosis and clinical management of individuals with Noonan Syndrome across Europe

Sixto García-Miñaúr[1], *Emma Burkitt-Wright*[2], *Alain Verloes*[3], *Guftar Shaikh*[4], *Jan Lebl*[5], *Ingegerd Östman-Smith*[6], *Cordula Wolf*[7], *Eduardo Ortega Castelló*[8], *Marco Tartaglia*[9], *Martin Zenker*[10], *Thomas Edouard*[11]

[1]*Institute of Medical and Molecular Genetics (INGEMM), Hospital Universitario La Paz Research Institute (IdiPAZ), Madrid, Spain,* [2]*Manchester Centre for Genomic Medicine, Manchester University NHS Foundation Trust and University of Manchester, Manchester, United Kingdom,* [3]*Department of Genetics, Hospital Robert Debré, Assistance Publique des Hopitaux de Paris (AP-HP), Paris, France,* [4]*Department of Paediatric Endocrinology, Royal Hospital for Children, Glasgow, United Kingdom,* [5]*Department of Pediatrics, Charles University, 2nd Faculty of Medicine, Prague, Czech Republic,* [6]*Institute of Clinical Sciences, Sahlgrenska Academy, Gothenburg University, Gothenburg, Sweden,* [7]*Department of Pediatric Cardiology and Congenital Heart Diseases, German Heart Center Munich at the Technical University Munich, Munich, Germany,* [8]*Department of Statistics and Data Science, Faculty of Statistical Studies, Complutense University of Madrid, Madrid, Spain,* [9]*Genetics and Rare Diseases Research Division, Ospedale Pediatrico Bambino Gesù, IRCCS, Rome, Italy,* [10]*Institute of Human Genetics, University Hospital Magdeburg, Magdeburg, Germany,* [11]*Endocrine, Bone Diseases, and Genetics Unit, Children's Hospital, Toulouse University Hospital, Toulouse, France.*

Introduction: Noonan syndrome (NS) is a developmental disorder caused by Ras/MAPK signalling pathway gene variants, with variable features including cardiac defects, short stature, distinctive facial appearance, skeletal abnormalities, variable cognitive deficits, and predisposition to certain cancers. Little is known about differences in the diagnosis and clinical management of individuals with NS across Europe.

Materials and Methods: The European Medical Education Initiative on NS developed a 60-question clinical practice survey to examine the diagnosis and clinical management of NS across Europe. Physicians from specialities particularly involved in the management of these patients (clinical geneticists, paediatric

endocrinologists, paediatric cardiologists) were invited via SurveyMonkey, with support from several European societies. Differences between specialties and countries were statistically assessed.

Results: Data were analysed from 364 respondents from 20 European countries. Most respondents came from France (20.9%), Spain (17.6%), Italy (15.4%), Germany (15.7%), UK (8.2%) and the Czech Republic (5.8%). Respondents were evenly distributed across clinical genetics (29.7%), paediatric endocrinology (40.1%) and paediatric cardiology (30.2%). A high degree of concordance about care practices was apparent across participating countries. While delayed diagnosis did not emerge as a critical issue, unmet needs regarding transition and increased awareness of family support groups were identified as common challenges.

Conclusion: Results from this survey provide a comprehensive diagnosis and clinical management overview for patients with NS across Europe. Work is ongoing to further analyse the results to identify key targets for improvement of patient care. The initiative was supported by an unrestricted grant from Novo Nordisk Europe A/S.

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P11.093.C A systematic review of lymphatic disorders in Noonan Syndrome:

*Jos Draaisma[1], **Lotte Kleimeier**[1], Julia Sleutjes[1], Erika leenders[2], Willemijn Klein[1]*

[1]Rradboudumc Amalia children's hospital, Nijmegen, Netherlands, [2]Rradboudumc, Nijmegen, Netherlands.

Introduction: This review aims to give an overview of the prevalence and clinical presentation of lymphatic disorders in genetically proven Noonan syndrome.

Methods: Literature search through Pubmed and EMBASE was performed until May 2020. All articles that included one or more genetically proven RASopathy and described the lymphatic phenotype were included. Quality assessment was performed using checklists developed by the Joanna Briggs Institute. Cohorts were combined to give insight in the prevalence, case reports and case series were used to analyze the clinical presentation and severity of these abnormalities.

Results: Searches through Pubmed and EMBASE resulted in 451 and 608 publications, respectively. In total, 59 publications could be included. Among these articles were 23 case reports, 17 case series and 19 cohort studies. The prevalence of lymphatic disorders was described in 14 cohorts. Prenatal lymphatic anomalies had a prevalence of 25% for increased NT, 19% for pleural effusions and 33% for cystic hygroma in Noonan Syndrome. The prevalence of postnatal lymphatic disorders caused by pathogenic variants in *RIT1* was 32.6%, caused by pathogenic variants in *SOS1* 44.4% and in pathogenic *PTPN1* variants 16.5%.

Conclusion: Based on the available published literature about genetically proven Noonan Syndrome, it appears likely that the lifetime prevalence of lymphatic disorders in Noonan syndrome is more than the generally accepted 20%.

J. Draaisma: None. **L. Kleimeier:** None. **J. Sleutjes:** None. **E. leenders:** None. **W. Klein:** None.

P11.094.D Lymphatic problems in patients with Noonan Syndrome

*Jos Draaisma[1], **Jessie Swarts**[2], Lotte Kleimeier[1], Erika Leenders[3], Willemijn Klein[1]*

[1]Rradboudumc Amalia children's hospital, Nijmegen, Netherlands, [2]Radboudumc Amalia children's hospital, Nijmegen, Netherlands, [3]Rradboudumc, Nijmegen, Netherlands.

Introduction: Noonan syndrome (NS) has been associated with an increased risk of lymphatic problems. An estimated prevalence of 20% has commonly been reported, however, cohort studies are scarce. The prevalence of lymphatic problems seem to differ between genes. Therefore, this study provide an overview of the clinical presentation and prevalence of lymphatic problems in patient with NS and Noonan-like syndromes. In addition, genotype-phenotype correlations will be investigated.

Methods: This retrospective cohort study included patients from the Radboud University Medical Center, who were clinically and genetically diagnosed with NS or Noonan-like syndrome till September 2020.

Results: The study population consisted of 267 patients, with a median age of 18 years (IQR: 9-34). Prenatal lymphatic problems most often presented as an increased nuchal translucency and/or chylothorax. Prenatal lymphatic problems increased the risk of lymphatic problems during infancy (odds ratio 10.5, 95% confidence interval 3.6-30.1). Postnatal lymphatic problems most often presented as lymphedema. The lifetime prevalence of lymphatic problems was 21%. Genotype-phenotype correlations showed a higher lifetime prevalence in patients with a *SOS2* or *SHOC2* variants compared to NS patients with a *PTPN11* variant (NS *PTPN11* vs *SOS2*: $p = 0.01$; NS *PTPN11* vs *SHOC2*: $p = 0.02$).

Conclusion: Prenatal and postnatal lymphatic problems are common in NS and Noonan-like syndrome patients. Patients with prenatal lymphatic problems had an increased risk of lymphatic problems during infancy. An especially high lifetime prevalence of lymphatic problems was found in patients with a *SOS2* or *SHOC2* variant.

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P11.095.A Clinical and molecular characterization of a group of Spanish and German patients with Noonan syndrome

Vanesa López González[1], *Mary Ballesta-Martínez*[1], *M. José Sánchez-Soler*[1], *Ana Teresa Serrano-Antón*[1], *Guillermo Glover-López*[2], *Begoña Ezquieta-Zubicaray*[3], *Alma Küchler*[4], *Beate Albrecht*[4], *Dagmar Wieczorek*[5], *Esther Zorio*[6], *Christina Lissewski*[7], *Martin Zenker*[7], *Encarna Guillén-Navarro*[1]

[1]*Sección de Genética Médica. Servicio de Pediatría. Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, Spain,* [2]*Centro de Bioquímica y Genética Clínica. Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, Spain,* [3]*Laboratorio de Diagnóstico Molecular. Hospital General Universitario Gregorio Marañón, Madrid, Spain,* [4]*5Institut für Humagenetik, Universtitätsklinikum Essen, Essen, Germany,* [5]*Institüt für Humangenetik, Universitätsklinikum Düsseldorf, Düsseldorf, Germany,* [6]*Servicio de Cardiología. Hospital Universitari i Politècnic La Fe, Valencia, Spain,* [7]*Institut für Humangenetik, Universitätsklinikum Magdeburg, Magdeburg, Germany.*

Introduction: Noonan syndrome represents one of the most prevalent disorders of multiple congenital anomalies.**Objective**. Clinical and molecular characterization of patients with NS. Estimation of its prevalence and genotype-phenotype correlation analysis.

Material and methods: Retrospective, descriptive and international collaborative study. Review of medical records of patients with a molecularly confirmed NS diagnosis.

Results: 88 cases. Main referral units: General Pediatrics and Pediatric Cardiology. Mean age at referral: 12.2 years. Estimated prevalence for NS: 1/6054 live newborns. Main findings: heart defects (76%), short stature (53%), microcephaly (40%) and intellectual disability (ID) (35%). All those classified as typical met van der Burgt diagnostic criteria compared to 75% of the atypical ones ($p = 0.003$). Association between low weight, short stature, microcephaly, motor delay and cardiac surgery with ID, also between central nervous system abnormalities and seizures. PTPN11 was the most frequently mutated gene (74%). Higher percentage of microcephaly in PTPN11 and hypertrophic cardiomyopathy in RAF1. Neurodevelopment and height were less affected in SOS1.

Conclusions: We present a wide series of NS cases with clinical manifestations, mostly, in accordance with previous publications. There is a large prevalence of microcephaly, higher in PTPN11, associated with ID and behavioral alteration, not previously reported. There appears to be greater severity of systemic involvement accompanying the severity of the craniofacial phenotype. The estimated prevalence in Murcia is lower than previously reported, the development of strategies to improve its detection being necessary. Greater awareness of adult specialties towards this group of diseases is necessary.

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P11.096.B Typical clinical diagnosis and negative first-line molecular results: when genome sequencing and transcriptomics integration helps untangle unexplained rare Mendelian diseases

Estelle Colin[1,2], *Yannis Duffourd*[1], *Patrick Callier*[1,3], *Emilie Tisserant*[1], *Thomas Besnard*[4,5], *Alice Goldenberg*[6], *Benjamin Cogné*[4,5], *Bertrand Isidor*[4,5], *Arthur Sorlin*[1,7], *Sébastien Moutton*[1,7], *Julian Delanne*[1,7], *Ange-Line Bruel*[1,3], *Frédéric Tran Mau-Them*[1,3], *Anne-Sophie Denommé-Pichon*[1,3], *Mélanie Fradin*[8], *Christel Dubourg*[9,10], *Magali Gorce*[2], *Salima El chehadeh*[11], *François-Guillaume Debray*[12], *Martine Doco Fenzy*[13,14], *Kévin Uguen*[15], *Anne Boland*[16], *Robert Olaso*[16], *Jean-François Deleuze*[16], *Damien Sanlaville*[15,17], *Christophe Philippe*[1,3], *Christel Thauvin-Robinet*[1,3,7], *Laurence Faivre*[1,7,18], *Antonio Vitobello*[1,3]

[1]*UFR Des Sciences de Santé, INSERM-Université de Bourgogne UMR1231 GAD « Génétique des Anomalies du Développement », FHU-TRANSLAD, Dijon, France,* [2]*Service de Génétique Médicale, CHU d'Angers, Angers, France,* [3]*Unité Fonctionnelle Innovation en Diagnostic Génomique des Maladies Rares, FHU-TRANSLAD, CHU Dijon Bourgogne, Dijon, France,* [4]*CHU Nantes, Service de Génétique Médicale, Nantes, France,* [5]*INSERM, CNRS, UNIV Nantes, CHU Nantes, l'institut du thorax, Nantes, France,* [6]*Department of Genetics and Reference Center for Developmental Disorders, Normandy Center for Genomic and Personalized Medicine, Normandie Univ, UNIROUEN, Inserm U1245 and Rouen University Hospital, Rouen, France,* [7]*Centre de Référence Maladies Rares Anomalies du Développement et syndromes malformatifs, Centre de Génétique, FHU-TRANSLAD, CHU Dijon, Dijon, France,* [8]*CHU Rennes, Service de Génétique Clinique, Centre de Référence Maladies Rares CLAD-Ouest, Rennes, France,* [9]*Service de Génétique Moléculaire et Génomique, CHU, Rennes, Rennes, France,* [10]*Univ Rennes, CNRS, IGDR, UMR 6290, Rennes, France,* [11]*Service de Génétique Médicale, Hôpital de Hautepierre, CHU Strasbourg, Strasbourg, France,* [12]*Metabolic Unit-Department of Medical Genetics, CHU & University Liège Domaine L Sart-Tilman Bât B35, Liège, Belgium,* [13]*Université de Reims Champagne-Ardenne, Reims, Reims, France,* [14]*Service de Génétique, CHU Reims, Reims, France,* [15]*Department of Genetics and Reference Center for Developmental Disorders, Lyon University Hospital, Groupement Hospitalier Est, Hospices Civils de Lyon, Lyon, France,* [16]*Université Paris-Saclay, CEA, Centre National de Recherche en Génomique Humaine, Evry, France,* [17]*GENDEV Team, CRNL, INSERM U1028 CNRS UMR5292 UCBL1, Lyon, France,* [18]*Institut GIMI, CHU de Dijon, Hôpital d'Enfants, Dijon, France; Oncogénétique, Centre de Lutte contre le Cancer Georges François Leclerc, Dijon, France.*

Developmental disorders are extremely heterogeneous, but typical clinical features reminiscent of recognizable OMIM syndromes, associated with well-known causative genes, represent a valuable help in the identification of causative genetic variants. However, first-line genetic investigations such as targeted sequencing, gene panel or exome sequencing may fail to reach a molecular diagnosis, resulting in a multiplication of diagnostic tests.

We recruited 15 individuals with typical clinical diagnosis of OMIM diseases with autosomal recessive mode of inheritance and a single heterozygous variant (9/15), or with X-linked recessive or autosomal dominant inheritance and no causative variant (6/15), identified by first-line genetic tests. We applied a two-step analysis including singleton genome sequencing (GS) complemented with transcriptomics analysis in blood or fibroblasts samples when required.

GS alone identified five causal single nucleotide variants (SNVs)/indels, four of which missed by first-line targeted tests, and four structural variants (including two deletions (6,5 kb and 4,2 kb), one balanced translocation and one complex rearrangement). mRNA-seq analysis permitted to validate the deleterious outcome of two complex rearrangements detected by GS and also find one structural variant and one deep intronic SNV not previously identified by GS alone.

We show that typical OMIM disorders with negative or inconclusive first-line results can be solved by a sequential deployment of GS and mRNA-seq. These approaches allowed us to confirm a molecular diagnosis in 87% (13/15) of our cohort. Overall, GS and mRNA-seq can be integrated in the diagnostic routine, allowing to establish new molecular diagnoses not otherwise identifiable by standard approaches.

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P11.097.C Contribution of genomic copy-number variations in a Brazilian cohort of syndromic orofacial clefts

*Rosana M. Candido-Souza[1], Roseli M. Zechi-Ceide[1], Nancy M. Kokitsu-Nakata[1], Camila W. Alvarez[1], Siulan Vendramini-Pittoli[1], Juliana F. Mazzeu[2], Hubertus J. Eussen[3], Annelies de Klein[3], **Fernanda S. Jehee**[3]*

[1]Department of Clinical Genetics, Hospital for Rehabilitation of Craniofacial Anomalies (HRCA), University of São Paulo, Bauru, Brazil, [2]Federal University of Brasília, Brasília, Brazil, [3]Department of Clinical Genetics, Erasmus University Medical Center, Rotterdam, Netherlands.

Background: Orofacial clefts represent ~30% of all congenital malformations being 30% of cleft lip with or without cleft palate and 50% of cleft palate cases syndromic. Chromosomal abnormalities, mainly in chromosomes 13, 18 and 22, are found in 11 to 23% of syndromic cases. Incomplete penetrance and the of investigation in large cohorts make it difficult to identify other chromosomal regions highly related to this malformation. **Aims**: Investigate the contribution of CNVs in syndromic orofacial clefts and identify new regions and genes herewith associated.

Methods: 62 syndromic orofacial clefts patients were analyzed using MLPA for subtelomeric and common microdeletion/microduplication syndromes. Patients with normal or inconclusive results were analyzed with microarray.

Results: MLPA detected six abnormalities (9.7%): two deletion 22q11.21, two unbalanced translocations (dup 3p/del 7q; del 4p/dup 11p) and two terminal deletions (del 18p; del18q). Microarray showed pathogenic CNVs in 11 patients (17.7%): deletions at 1q, 16p11.2, 18q, 19p13.2, distal 22q11.21 and Xp11; duplications at 15q13 and 18q12 and a complex rearrangement in chromosome 13. One patient had a deletion at *NRXN1* and six patients (9.7%) presented variants of unknown significance. A strong suggestion of consanguinity was found in 8.9% of the cases.

Conclusion: Genomic microrearrangements (27.4%) play an important role in syndromic orofacial clefts. We detected CNVs in regions not frequently related to oral clefts (1q, 15q13, 16p11) and describe cleft lip in Malan syndrome (19p13.2). Consanguinity plays an important role in onset of orofacial clefts, by increasing the risk of a recessive disease or incrementing risk alleles.

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P11.098.D A gain of one copy at band 14q22.3q23.1 that involves the *OTX2* gene is implicated in hemifacial microsomia

***Raluca Oancea-Ionescu**[1,2], Clara Herrero-Forte[1,2], Maria Fenollar-Cortés[1,2], Enrique Criado-Vega[1,3], Maria Calvente -Garcia[4], Dan Diego-Álvarez[5], Carmen Cotarelo-Pérez[1,2]*

[1]Hospital Clinico San Carlos, Madrid, Spain, [2]Unidad de Genética Clínica, Servicio de Análisis Clínicos, Instituto de Medicina del Laboratorio, Madrid, Spain, [3]Servicio de Neonatología, Instituto del Niño y Adolescente., Madrid, Spain, [4]Nimgenetics New Integrated Medical Genetics, Madrid, Spain, [5]Sistemas Genómicos Grupo Biomédico ASCIRES, Valencia, Spain.

Introduction: The hemifacial microsomia (HMF) is a heterogeneous genetic disorder affecting the development of the structures derived from the first and second branchial arches such the jaw, the buccal structures and the hearing system.

Material and methods: The patient is a 7-month-old female who presents the following clinical manifestations: right HMF; left preauricular tag; microtia and absence of auditory canal in the right ear with hearing loss; absence of right ascending ramus and mandibular body; right zygomatic arch hypoplasia with deviation of the mouth commissure; mild micrognathia; normal palate and an interventricular communication spontaneously closed. Cerebral ultrasound was normal. X-ray showed no vertebral abnormalities. Next-generation sequencing using a virtual panel of 55 genes for HMF and/or preauricular tags and array-CGH were performed.

Results: Both tests detected a de novo copy number variation consisting in a pathogenic interstitial gain of one copy at band 14q22q23.1, which is at least 1,33 megabases. It involves four OMIM genes, of which the *OTX2* stands out because its duplication was previously associated with HMF as it is shown in following table:

Conclusions: This result and the previous cases suggest that the gain of one copy at band 14q22.3q23.1 is one of the etiological region of HMF and the *OTX2* is the most likely causal gene. Determining the HFM etiology allows an accurate diagnosis and offers reproductive and familial genetic advice.

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P11.099.A PACS2, PACS1 and VACTERL a clinical overlap

***Hannah Massey**, John Dean, Dawn O'Sullivan, Stephen Tennant*

Aberdeen royal infirmary, Aberdeen, United Kingdom.

Whole exome sequencing (WES) has led to the discovery of new genes involved in developmental delay. Two of these are the evolutionary linked proteins PACS1 and PACS2, which function as metabolic switches. We present a case of patient with the previously described PACS2 c.624G>A; p.Glu209Lys variant. However, while our patient has the infantile epilepsy, developmental delay and cerebella hypoplasia previously described, he also had novel features. This included anal atresia, Tetralogy of Fallot and vertebral abnormalities, meaning he was previously diagnosed with VACTERL. Cardiac abnormalities are more commonly seen in PACS1 variants and this case strengthens the phenotypic similarities between the two conditions. There are plausible genetic mechanisms causing the cardiac and anal anomalies seen in our patient and suggest the PACS2 disease spectrum should be expanded.

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P11.101.C Low risk of embryonal cancer in *PIK3CA*-Related Overgrowth Spectrum: impact on screening recommendations

Laurence Faivre[1,2], *Jean-Charles Crépin*[1,3,4], *Manon Réda*[5], *Sophie Nambot*[5], *Virginie Carmignac*[1,4], *Caroline Abadie*[6], *Audrey Putoux*[7], *Juliette Mazereeuw-Hautier*[8], *Aude Maza*[8], *Maxime Luu*[9], *Yannis Duffourd*[1], *Christophe Philippe*[1], *Christel Thauvin-Robinet*[1], *Martin Chevarin*[1], *Claire Abasq-Thomas*[10], *Jeanne Amiel*[11], *Stéphanie Arpin*[12], *Geneviève Baujat*[13], *Didier Bessis*[14], *Emmanuelle Bourrat*[15], *Odile Boute*[16], *Anne-Claire Bursztejn*[17], *Nicolas Chassaing*[18], *Christine Coubes*[19], *Patrick Edery*[7], *Salima El Chehadeh*[20], *Alice Goldenberg*[21], *Smail Hadj-Rabia*[22], *Damien Haye*[23], *Bertrand Isidor*[24], *Marie-Line Jacquemont*[25], *Didier Lacombe*[26], *Bruno Leheup*[27], *Ludovic Martin*[28], *Anabel Maruani*[29], *Fanny Morice-Picard*[26], *Florence Petit*[16], *Alice Phan*[30], *Lucille Pinson*[19], *Eve Puzenat*[31], *Massimiliano Rossi*[7], *Renaud Touraine*[32], *Clémence Vanlerberghe*[16], *Marie Vincent*[24], *Catherine Vincent-Delorme*[16], *Sandra Whalen*[33], *Marjolaine Willems*[19], *Tristan Mirault*[34], *Paul Kuentz*[1,35], *Pierre Vabres*[1,3,4,2]

[1]*Equipe INSERM UMR1231, Génétique des Anomalies du Développement, Université Bourgogne Franche-Comté, Dijon, France,* [2]*Fédération Hospitalo-Universitaire Médecine Translationnelle et Anomalies du Développement, CHU Dijon Bourgogne, Dijon, France,* [3]*Service de Dermatologie, CHU Dijon Bourgogne, Dijon, France,* [4]*Centre de référence Maladies Rares Génétiques à Expression Cutanée (MAGEC), CHU Dijon, Dijon, France,* [5]*Service de génétique, CHU Dijon Bourgogne, Dijon, France,* [6]*Département d'Oncologie Médicale, Saint-Herblain, France,* [7]*Département de Génétique, Hospices Civils de Lyon, Lyon, France,* [8]*Service de Dermatologie, CHU Toulouse, Toulouse, France,* [9]*Centre d'Investigation Clinique – module pluri-ithématique, CHU Dijon Bourgogne, Dijon, France,* [10]*Département de Pédiatrie et Génétique Médicale, CHU Brest Morvan, Brest, France,* [11]*Service de Génétique Médicale, Hôpital Necker Enfants Malades, Paris, France,* [12]*Service de Génétique Clinique, CHRU de Tours, Tours, France,* [13]*Service de Génétique Médicale, Hôpital Necker-Enfants Malades, Paris, France,* [14]*Département de Dermatologie, CHRU de Montpellier, Montpellier, France,* [15]*Service de dermatologie, CHU St-Louis Lariboisière, Paris, France,* [16]*Service de Génétique Clinique, CHU Lille, Lille, France,* [17]*Service de Dermatologie, CHU de Nancy, Nancy, France,* [18]*Service de Génétique Médicale, CHU Toulouse, Toulouse, France,* [19]*Département de Génétique Médicale, Maladies rares et Médecine Personnalisée, CHRU de Montpellier, Montpellier, France,* [20]*Service de Génétique Médicale, CHU de Strasbourg, Strasbourg, France,* [21]*Service de Génétique, CHU de Rouen et Centre Normand de Génomique Médicale et Médecine Personnalisée, Rouen, France,* [22]*Service de Dermatologie et Centre de Référence des Maladies Rares Génétiques à Expression Cutanée (MAGEC), Université Paris Descartes-Sorbonne Paris Cité, Institut Imagine, Hôpital Universitaire Necker-Enfants Malades, Paris, France,* [23]*Service de Pédiatrie, CHU de Tours, Tours, France,* [24]*Service de Génétique Médicale, CHU de Nantes, Nantes, France,* [25]*Unité de Génétique Médicale, CHU de la Réunion, Saint Pierre, France,* [26]*Service de Génétique Médicale, CHU de Bordeaux, Bordeaux, France,* [27]*Service de Génétique Clinique, CHU de Nancy, Nancy, France,* [28]*Service de Dermatologie, CHU d'Angers, Angers, France,* [29]*Service de Dermatologie, Unité de Dermatologie Pédiatrique, Tours, France,* [30]*Service de Dermatologie, CHU de Lyon, Lyon, France,* [31]*Service de Dermatologie, CHU Besançon, Besançon, France,* [32]*Service de Génétique Clinique, CHU de Saint-Etienne, Saint-Etienne, France,* [33]*Unité Fonctionnelle de Génétique Clinique, Hôpital Armand-Trousseau, Paris, France,* [34]*Centre de référence des maladies vasculaires rares, Centre Européen Georges Pompidou, Paris, France,* [35]*Génétique Biologique Histologie, CHRU de Besançon, Besançon, France.*

Introduction: The *PIK3CA*-Related Overgrowth Spectrum (PROS) encompasses various conditions caused by mosaic activating *PIK3CA* mutations. *PIK3CA* somatic mutations are frequently involved in various cancer types. Some overgrowth syndromes are associated with an increased risk of Wilms' tumour (WT) warranting screening. In PROS, abdominal ultrasound monitoring every 3 months has been discussed for the risk of WT. We aimed to determine the risk of cancer in patients with confirmed PROS to evaluate the relevance of screening recommendations.

Patients and methods: The development of a cancer was searched within 267 PROS patients. A literature review of cancer reports in PROS was performed.

Results: Median age at last visit was 11.2 years (range 4 months-68 years). Five patients developed cancer (1.9%): nephroblastoma at age 9; granulosa juvenile tumor at age 16; gastric adenocarcinoma at age 52 ; myelodysplasic syndrome at 55 and basocellular carcinoma at 59 in the same patient; clear cell renal carcinoma at age 38. In the literature, 6 cases of WT (0.12%) and 4 cases of other cancers have been reported out of 483 *PIK3CA* patients, carying p.His1047Leu/Arg in particular (15 patients with WT, 40 with other cancer out of 820 patients with no molecular confirmation). A focus has been given to the location of the hypertrophy.

Discussion: The risk of WT in PROS appears lower than 5%, therefore with insufficient evidence to recommend routine abdominal imaging. Long-term follow-up studies are needed to conclude for other cancer types, as well as a relationship with the extent of tissue mosaicism.

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P11.102.D A 3,195 kb duplication at 2q14.3 in a proband with a t(17;19)(p13.1;p13.3)mat is most likely associated with craniofacial dimorphisms, *developmental and neurological anomalies*

Natália Oliva-Teles[1,2,3], *Mariana Marques*[4], *Manuela Mota-Freitas*[1,2], *Cristina Candeias*[1,2], *Joana Fino*[4], *Ana Fortuna*[1,2], *Dezso David*[4]

[1]*Centro de Genética Médica Doutor Jacinto Magalhães/Centro Hospitalar Universitário do Porto, Porto, Portugal,* [2]*Unit for Multidisciplinary Research in Biomedicine, Institute of Biomedical Sciences Abel Salazar, University of Porto (UMIB/ICBAS/UP), Porto, Portugal,* [3]*Departamento de Medicina da Comunidade Informação e Decisão em Saúde, Faculty of Medicine, University of Porto, Porto, Portugal,* [4]*National Health Institute "Doutor Ricardo Jorge", Lisbon, Portugal.*

Introduction: Characterization of naturally occurring, cytogenomically visible or cryptic structural variants associated with human disorders is important for identifying pathogenic alterations that otherwise could be difficult or impossible to identify.

Case Report/Methods: Proband DGRC0021 presents with a familial apparently balanced t(17;19)(p13.1;p13.3)mat, craniofacial dysmorphisms, global developmental delay and aggressive behavior. Her carrier mother and grandmother were phenotypically normal, whereas her father died of a cerebral aneurysm rupture in his late 20s. Long-insert genome and Sanger

sequencing were performed for identifying structural variants in their responsible for the clinical phenotype.

Results: The 17p13.1 breakpoint at g.9,819,770 disrupts IVS 1 of *GSG1L2*, whereas the 19p13.3 breakpoint at 6,573,218, is within a low-complexity region. Based on deletions at the breakpoint regions the seq[GRCh38/hg38] t(17;19)(19pter→19p13.3::17p13.1→17qter;17pter→17p13.1::19p13.3→19qter)mat is considered unbalanced. Neither the disrupted gene nor those localized in the disrupted topologically associated domains (TADs) explained the reported phenotype. Subsequently a 3,195 kb duplication was identified at 2q14.3. Two genes associated with autosomal dominant disorders, *PROC* (OMIM*612283) and *HS6ST1* (OMIM*604846) are localized within the duplicated region. This alteration is either paternally inherited or *de novo*.

Conclusions: We showed that the translocation is nonpathogenic and identified a most likely pathogenic paternally inherited duplication. To our knowledge, this is the first reported case leading to duplication of *PROC*. While the pathogenic effect of *PROC* deficiency is well known, the effect of a possible duplication of *PROC* is unknown. Nevertheless, we propose that the clinical phenotypes in the proband and her father are caused by this duplication.

N. Oliva-Teles: None. **M. Marques:** None. **M. Mota-Freitas:** None. **C. Candeias:** None. **J. Fino:** None. **A. Fortuna:** None. **D. David:** None.

P11.103.A Implementation and assessment of a rapid Whole Exome Sequencing protocol in paediatric patients admitted to intensive care units or highly complex paediatric units in a tertiary hospital of the Spanish National Health System

Marta Pacio Miguez[1], *Sixto García-Miñaúr*[1,2,3], *Ángela del Pozo*[1,2], *Juan José Menéndez Suso*[4], *Francisco J. Climent Alcalá*[5], *Patricia Álvarez García*[6], *María Sánchez Holgado*[6], *Carmen Jiménez Rodríguez*[1], *Juan Manuel Montejo*[1], *Rocío Mena*[1], *Sofía Siccha*[7], *Mario Solís*[1], *Victoria Eugenia Fernandez Montaño*[1], *Mª Victoria Gomez del Pozo*[1], *Natividad Gallego Onís*[1], *María Esther Rubio Martín*[1], *Pablo Lapunzina*[1,2,3], *Sonia Rodríguez Novoa*[1], *Fernando Santos-Simarro*[1,2,3], *María Palomares-Bralo*[1,2,3]

[1]*Instituto de Genética Médica y Molecular, INGEMM, Madrid, Spain,* [2]*CIBERER, Centro de Investigación Biomédica en Red de Enfermedades Raras, ISCIII, Madrid, Spain,* [3]*European Reference Network, ERN ITHACA, Madrid, Spain,* [4]*Servicio de Cuidados Intensivos Pediátricos, Hospital Universitario La Paz, Madrid, Spain,* [5]*Unidad de Patología Compleja pediátrica, Hospital Universitario La Paz, Madrid, Spain,* [6]*Unidad de cuidados intensivos neonatales, Hospital Universitario La Paz, Madrid, Spain,* [7]*Servicio de pediatría, Hospital Universitario La Paz, Madrid, Spain.*

Introduction: Genetic disorders contribute significantly to the mortality and morbidity of affected paediatric patients, requiring prolonged admissions in NICU, PICU or complex hospitalization facilities. Early identification of the genetic cause favour the efficient management of these children, provide prognostic information and allow genetic counselling of families. We have tested and set up a rapid workflow method for achieving a genetic diagnosis through rapid whole exome sequencing (rWES) in critically ill or children with high complexity with a suspected genetic disorder in the environment of a tertiary paediatric hospital of the Spanish National Health System.

Materials And Methods: Over the last year, our working group identified and implemented the clinical and laboratory needs to achieve a genetic diagnosis through rWES. Thirteen children meeting the inclusion criteria were studied using a trio approach.

Results: We found a causative variant in genes known to be associated with developmental disorders in 6 of the 13 cases (46%). Additionally, we found variants of potential research interest in 3 cases. Mean time for achieving a genetic result was 30,8 days (median = 30, std = 9.2) after enrolment in the study.

Conclusions: We have set up a workflow to assess the impact of rWES in paediatric patients admitted to NICU, PICU or complex paediatric unit. A genetic result was achieved in a mean time of 30,8 days. The diagnostic and clinical utility is verified by a diagnostic rate of 46%. Impact in patient management and costs derived from this protocol during the first year of implementation will be discussed.

M. Pacio Miguez: None. **S. García-Miñaúr:** None. **Á. del Pozo:** None. **J. Menéndez Suso:** None. **F. Climent Alcalá:** None. **P. Álvarez García:** None. **M. Sánchez Holgado:** None. **C. Jiménez Rodríguez:** None. **J. Manuel Montejo:** None. **R. Mena:** None. **S. Siccha:** None. **M. Solís:** None. **V. Fernandez Montaño:** None. **M. Gomez del Pozo:** None. **N. Gallego Onís:** None. **M.E. Rubio Martín:** None. **P. Lapunzina:** None. **S. Rodríguez Novoa:** None. **F. Santos-Simarro:** None. **M. Palomares-Bralo:** None.

P11.104.B Spectrum of mutations in Ras-MAPK pathway in Russian probands

Anna Orlova, *Alexander Polyakov, Polina Gundorova, Oksana Ryzhkova*

Clinical phenotype comparisons between our patient and the previous cases with gain of one copy at band 14q22q23.1 that include *OTX2* gene

	Our patient	Ballesta-Martínez MJ, 2013PMID: 23794319						Zielinski D, 2014PMID: 24816892					Ehrenberg M,2019PMID: 31814694	
Family relationship	**Index case**	**Index case**	**Father**	**Uncle**	**Cousin**	**Cousin ´s son**	**Cousin´s daughter**	**Maternal grand-mother**	**Maternal great-uncle**	**Mother**	**Brother**	**index case**	**Indexcase**	**Father**
Facial asymmetry	+	+	+	-	-	+	+	+	+	+	+	+	+	+
Facial cleft	-	+	+	-	-	+	-	+	+	+	+	+	-	ND
Temporo-mandibular joint abnormality	+	+	-	-	ND	ND	ND	+	+	+	+	+	-	ND
Ear constriction/cleft/ microtia	+	+	-	-	-	+	+	-	-	-	-	+	+	ND
Auricular pits	-	+	-	-	-	+	+	ND	ND	ND	ND	ND	-	ND
Pre- or post-auricular tags	+	+	+	+	+	+	+	+	-	+	+	+	-	ND
Stenoticnarrow ear canals	+	+	-	ND	ND	+	+	ND	ND	ND	ND	ND	-	ND
Abnormal palate	-	-	-	-	-	-	-	ND	ND	ND	ND	ND	-	ND
Micrognathia	+	+	-	-	-	-	-	-	+	-	+	+	+	ND
Hearing loss	+	+	-	-	ND	-	-	ND	ND	ND	ND	ND	+	ND

Federal State Budgetary Scientific Institution "Research Centre for Medical Genetics", Moscow, Russian Federation.

Introduction: Variants in genes related to the Ras-MAPK pathway cause diseases that form the large group of rasopathies. The most common syndrome in this group is Noonan syndrome. To date, the incidence of rasopathiesis 1-2 per 20000 newborns. It is difficult to clinically differentiate Rasopathies.

Materials and Methods: DNA of 308 unrelated probands with Rasopathies were analysed with a custom panel (23 genes) for new generation sequencing on Ion Torrent S5. The coverage was 91-100% of the major Ras-MAPK genes.

Results: Out of 308 probands, pathogenic and likely pathogenic variants were found in 121 cases. The results are shown in the table. Novel variants classified as VOUS were found in 5,2% cases and they require further research.

Gene	Number(percent) of pathogenic and Likely pathogenic variants	Number of mutations haven't been described earlier
PTPN11	63(52%)	1
SOS1	11(9%)	2
BRAF	11(9%)	
NF1	9(7.4%)	3
SHOC2	8(6.6%)	1
HRAS	4(3.3%)	
RIT1	3(2.5%)	
KRAS	3(2.5%)	1
MAP2K1	3(2.5%)	2
SPRED1	2(1.65%)	1
CBL	1(0.83%)	
LZTR1	1(0.83%)	2
NRAS	1(0.83%)	
RAF1	1(0.83%)	3

Conclusions: The proportions of diseases and mutation spectrum in the group of patients with Rasopathies from the Russia were determined. Most of the the cases (52%) are due to mutations in the *PTPN11* gene, followed by the frequency of *SOS1*, *BRAF*, *NF1*, *SHOC2* and others. This data is comparable with the incidence in other populations. It was not possible to identify the cause of the disease in 61% of cases.

A. Orlova: None. **A. Polyakov:** None. **P. Gundorova:** None. **O. Ryzhkova:** None.

P11.105.C Risk of autoimmune diseases in patients with RASopathies: systematic study of humoral and cellular immunity

Maria Anna Siano[1], *Mariateresa Falco*[2], *Valeria Marchetti*[3], *Stefano Pagano*[3], *Francesca Di Candia*[3], *Daniele De Brasi*[4], *Alessandro De Luca*[5], *Valentina Pinna*[5], *Simona Sestito*[6], *Daniela Concolino*[6], *Marco Tartaglia*[7], *Pietro Strisciuglio*[3], *Vittoria D'Esposito*[8], *Serena Cabaro*[8], *Giuseppe Perruolo*[8], *Pietro Pietro Formisano*[8], *Daniela Melis*[1]

[1]*Postgraduate School of Pediatrics, Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, Salerno, Italy,* [2]*Pediatric Unit, San Giovanni di Dio e Ruggi d'Aragona University Hospital, Salerno, Italy,* [3]*Postgraduate School of Pediatrics, Faculty of Medicine University of Naples Federico II, Naples, Italy,* [4]*Unit of Pediatrics, AORN Santobono-Pausilipon, Naples, Italy,* [5]*Molecular Genetics Unit, Fondazione Casa Sollievo della Sofferenza, IRCCS, San Giovanni Rotondo, Foggia, Italy,* [6]*Department of Medicine, University "Magna Graecia", Catanzaro, Italy,* [7]*Genetics and Rare Diseases Research Division, Ospedale Pediatrico Bambino Gesù, Rome, Italy,* [8]*Department of Translational Medical Sciences, University of Naples Federico II & Institute of Endocrinology and Experimental Oncology, National Research Council, Naples, Italy.*

Introduction: Abnormalities of the immune system are rarely reported in patients with RASopathies. The aim of this study was to investigate the prevalence of immune system dysfunction in a cohort of patients affected by RASopathies.

Materials and Methods: A cohort of 69 patients and age- and sex-matched control group were enrolled. Autoimmune disorders were investigated according to international consensus criteria. Immune framework was also evaluated by immunoglobulin levels, lymphocyte subpopulations, autoantibodies levels and panel of inflammatory molecules, in both patients and controls.

Results: Frequent upper respiratory tract infections were recorded in 2 patients (2.89%), psoriasis was diagnosed in 1 (1.45%), as well as alopecia. Low IgA levels were detected in 8/44 patients (18.18%), low CD8 T cells in 13/35 (37.14%). Anti-tg and anti-TPO antibodies were detected in 3/24 patients (12.5%), anti r-TSH in 2 (8.33%), all in euthyroidism. All tested patients showed increased inflammatory molecules compared to controls. Serum IgA and CD8 levels were significantly lower in patients than in controls (p 0,00685; p 0,000656 respectively).

Conclusions. A major tendency to autoimmune phenomena than to immunodeficiency was recorded in patients as demonstrated by the finding of circulating autoantibodies, low levels of CD8 T cells and high levels of inflammatory cytokines. These findings may anticipate the detection of overt autoimmune disease. In order to recommend routine screening for autoimmunity in asymptomatic patients, continuous monitoring will be required for possible emergence of autoimmune disease.

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P11.106.D Novel mutation and report of two new physical findings in renpenning syndrome

Hande Kaymakcalan[1], *Adife Gulhan Ercan Sencicek*[2], *Ayse Nurcan Cebeci*[3], *Ali Seyfi Yalim Yalcin*[4]

[1]*Demiroglu Bilim University, Istanbul, Turkey,* [2]*Masonic Medical Research Institute, Utica, NY, USA,* [3]*Private practise, Erlangen, Germany,* [4]*Private practise, Istanbul, Turkey.*

Introduction: We report a novel deleterious hemizygous X chromosome variant in *PQBP1* (NM_001167989:p.Arg153fs) that leads to Renpenning syndrome (OMIM#309500).

Materials and methods: 17 years old Turkish male patient with tetrology of fallot and pulmonary atresia was seen due to dysmorphic features and short stature. Fish test was negative for DiGeorge Syndrome when he was 2 years old. Whole exome sequencing was done.

Results: On physical examination, his height was 149,4 cm (-3,92 SDS), weight was 38,2 kg (-4,43 SD). His body mass index was 17.11 kg/m^2 (-2,5 SD). His head circumference was 48 cm (-6,34 SD). He had long narrow face, bulbous nose, sparse lateral eye brows and strabismus. He had webbed neck. He had lean body build, muscular atrophy of upper back and scoliosis. He had

slender feet and overriding 5th right toe over 4th toe. This feature has not been described before. On genital examination his left testis could not be palpated in scrotum and in inguinal canal. His right testis was 20 cc. This feature has not been described before either. He also had mild intellectual deficiency. Unfortunately, he died due to endocarditis complication. Sanger sequencing confirmed that he had deleterious hemizygous X chromosome variant in *PQBP1* (NM_001167989:p.Arg153fs) inherited from an unaffected heterozygous mother. Three unaffected sisters all inherited the variant.

Conclusion: Patients with previous inconclusive genetic test results must be reevaluated. Diagnosis is important for management and genetic counselling. Grant: This work was supported by U54HG006504 (Yale Center for Mendelian Disorders, to MG).

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P11.107.A Unpredictable results of undiagnosed patients via exome sequencing: Ribosomopathies

Saide Betül Arslan Satılmış[1], *Busranur Cavdarli*[1], *Ahmet Cevdet Ceylan*[2], *Ebru Tuncez*[1], *Cavidan Nur Semerci Gündüz*[2]

[1]*Ankara City Hospital, Ankara, Turkey,* [2]*Ankara Yildirim Beyazit University, Ankara, Turkey.*

Introduction: Ribosomopathies caused by abnormalities of ribosomal proteins and on biogenesis factors are a broad spectrum that affects various tissues including the nervous system, bone marrow, and ectoderm or causes developmental-delay or cancer-susceptibility. Although the most common ribosomopathies are Diamond-Blackfan anemia, 5q-Syndrome, Shwachman-Diamond syndrome, Dyskeratosis Congenita, Cartilage hair hypoplasia, new diseases have been defining by the development of new technologies.

Material/Method: Five undiagnosed patients with growth retardation, epilepsy, cerebellar hypoplasia, hepatic dysfunction or metabolic disease were examined. Conventional-karyotype and microarray analyses were normal. Whole-exome-sequencing was performed via XGEN.

Results: The determined variants were evaluated according to the patients' clinical features. One pathogenic variant of the *EIF2B3* gene for a 7-year-old boy with leukodystrophy and one likely pathogenic variant of the *MRM2* gene for an 18-year-old male with hepatosplenomegaly and tremor were thought to be causative, respectively. Three variants of uncertain significance(VUS); in the *POLR3A* gene (3-year-old girl with cerebellar atrophy and epilepsy), *TAF6* gene(3-year-old boy with developmental delay and mental deficiency), and *PARN* gene(3-year-old girl with microcephaly and cerebellar vermis atrophy), were detected. Although these variants are VUS, they were thought to be clinically responsible in the light of segregation analyses in the family.

Conclusion: Ribosomopathies are a broad-spectrum of diseases those molecular basis has been better understood as the whole-exome-sequencing becomes viable. Therefore, the new mutations reported and their clinical relevance is important. The spectrum of ribosomopathies will be extended by reporting the new mutations in the genes on the ribosomal-pathway and by enlightening their reflections on the phenotypes.

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P11.108.B Novel missense variant in *CCDC22* causes X-linked Ritscher-Schinzel/3C syndrome: further evidence on the prenatal and postnatal clinical phenotype

Emanuela Ponzi, *Paola Orsini, Romina Ficarella, Antonia Lucia Buonadonna, Maria Fatima Antonucci, Mattia Gentile*

Di Venere Hospital-Unit of Medical Genetics, Bari, Bari, Italy.

Introduction: Ritscher-Schinzel Syndrome (RSS) is a rare developmental disorder characterized by intellectual disability, cerebellar/brain-malformations, congenital heart defects and craniofacial abnormalities.

Materials and Methods: We report a male infant with prenatal history of fetal ascites and postnatal typical dismorphic facial features, cerebellar vermis hypoplasia, enlarged cisterna magna and hepatomegaly. Karyotype and CGH-array were performed prenatally and whole-exome sequencing (WES), performed postnatally.

Results: Karyotype and CGH-array were normal. WES detected a novel maternal X-linked missense variant (c.49A>C; p.Thr17Pro) in exon 1 of the *CCDC22* gene. The variant, not previously reported, has been classified as uncertain/likely pathogenic (ACMG criteria). Allelic variant in the same position c.49A> G (p.Thr17Ala) is reported in ClinVar as pathogenic. *CCDC22* is a highly conserved and broadly expressed protein that has been shown to interact through its N-terminal part with COMMD proteins that take part in multiple processes including regulation of NF-kB signaling, copper export from the liver and sodium transport. Patients with RSS, c.49A>G in *CCDC22* gene and elevated serum copper and ceruloplasmin concentration have been previously reported. Recently, murine model study showed that COMMD protein deficiency leads to a defect in the transport of ATP7B (copper membrane trasporter) from cytosolic vescicles to the plasma membrane, resulting in hepatic copper accumulation. Serum and urinary copper and ceruloplasmin dosage are progress to test this hypothesis.

Conclusion: on the basis of the recent scientific evidence we speculate that the prenatal finding of ascites and the postnatal finding of hepatomegaly could be an additional clinical features of RSS.

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P11.110.D Truncating mutations in MAGEL2 cause alterations in Aβ1-40 levels and gene expression in fibroblasts

Laura Castilla-Vallmanya[1,2], *Mónica Centeno-Pla*[1,2], *Héctor Franco-Valls*[1,2], *Aina Prat-Planas*[1,2], *Elena Rojano*[3], *Pedro Seoane*[3,2], *James R. Perkins*[3,2,4], *Juan A. García-Ranea*[3,2,3], *Clara Oliva*[5], *Aida Ormazabal*[5,2], *Rafael Artuch*[5,2], *Raquel Rabionet*[1,2], *Daniel Grinberg*[1,2], *Susanna Balcells*[1,2], *Roser Urreizti*[1,2,5]

[1]*Department of Genetics, Microbiology and Statistics, Faculty of Biology, University of Barcelona, IBUB, IRSJD, 08028, Barcelona, Spain,* [2]*Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER)- Instituto de Salud Carlos III, Barcelona, Spain,* [3]*Department of Molecular Biology and Biochemistry, University of Malaga, 29010, Málaga, Spain,* [4]*Institute of Biomedical Research in Malaga (IBIMA), Málaga, Spain,* [5]*Clinical Biochemistry Department, Hospital Sant Joan de Déu, 08950, Esplugues de Llobregat, Spain.*

Introduction: Truncating mutations in *MAGEL2*, a gene mapping in the Prader-Willi region (15q11-q13), are associated with Schaaf-Yang syndrome (#MIM 6615547;SYS), a severe ultra-rare neurodevelopmental disease. SYS patients show a clinical phenotype partly overlapping the one typically observed in Prader-Willi syndrome patients (#MIM 176270;PWS). MAGEL2 contributes to the regulation of the retrograde transport and protein recycling pathways and previous studies show that impairment of VPS35, a

MAGEL2 partner in retromer function, lead to dysregulation of APP transport and cleavage.

Materials & Methods: In fibroblasts from SYS and PWS patients and healthy controls, we analysed the excretion levels of amyloid-β 1-40 peptide ($A\beta_{1-40}$). We also analysed targeted metabolomics patterns (by mass spectrometry) and gene expression (by mRNAseq). ExpHunter Suite was used to identify differentially expressed genes (DEGs) and co-expression modules and perform functional enrichment.

Results: We observed a significant decrease of $A\beta_{1-40}$ levels in the extracellular media of SYS fibroblasts compared to both PWS patients and controls. Preliminary results show small but statistically significant metabolomic alterations, being glycine especially relevant. We identified 132 DEGs, some of them related to mitotic mechanisms and extracellular matrix formation and organization.

Conclusions: $A\beta_{1-40}$ excretion level is a promising biomarker for SYS, and could help to better understand its pathophysiology. The identified DEGs in SYS fibroblasts suggest a potential novel role for MAGEL2 in mitosis and extracellular matrix homeostasis that should be further studied.**Funding:** Associació Síndrome Opitz C, Spain; Spanish Government (CIBERER -U720; PID2019-107188RB-C21, SAF2016-75948-R, FECYT-PRECIPITA); Catalan Government (PERIS SLT002/16/00174).

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P11.111.A The first two Portuguese patients with Schuurs-Hoeijmakers syndrome: case report and review of the literature

Susana Lemos Ferreira[1], *Pedro Maia Almeida*[2], *Catarina Silva Rosas*[2], *Mafalda Santos Melo*[1], *Sérgio Bernardo Sousa*[2], *Marta Amorim*[1], *Teresa Kay*[1]

[1]*Serviço de Genética Médica, Hospital Dona Estefânia, Centro Hospitalar e Universitário de Lisboa Central, Lisboa, Portugal,* [2]*Serviço de Genética Médica, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal.*

Introduction: Schuurs-Hoeijmakers Syndrome (SHS, OMIM #615009) is a rare cause of developmental delay with distinctive dysmorphic features. SHS is characterized by variable degrees of intellectual disability with language skills typically affected, hypotonia, epilepsy, behaviour issues, feeding difficulties, constipation, cryptorchidism, short stature, and structural malformations (cardiac, brain, ocular, kidney and skull anomalies). The majority of cases results from a *de novo* heterozygous pathogenic variant in *PACS1* c.607C>T p.(Arg203Trp). So far, only 52 individuals were reported in the literature. Here, we aim to better characterize SHS phenotype.

Methods: We inquired all Portuguese medical genetics departments, collected clinical data and compared with previous described cases.

Results: One patient, a 2-year-old male, presented with developmental delay, facial dysmorphisms, hypotonia, coloboma, cryptorchidism, plagiobrachycephaly and single umbilical artery. A second patient, a 4-year-old male, was referred for developmental delay, epilepsy, facial dysmorphisms, prenatal macrocephaly, congenital heart defect, cryptorchidism, umbilical hernia and prior history of constipation. In both cases, previous investigation was normal and molecular confirmation of SHS was obtained by exome sequencing that identified a *de novo* pathogenic variant in *PACS1* c.607C>T p.(Arg203Trp).The overall phenotypes were consistent with the reported cases, sharing the most frequent described clinical features such as facial dysmorphisms, intellectual disability, epilepsy, and congenital heart defects, but also less frequent features as umbilical hernia.

Conclusions: SHS is a distinct neurodevelopmental disorder with multiple congenital anomalies, and the identification of additional cases increases the current understanding of its clinical spectrum. To our knowledge, these cases represent the first molecular confirmed SHS patients in Portugal.

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P11.112.B Heterozygous mutation in SCRIB gene in adult patient with myelomeningocele and primary infertility

Zohor Asaad Azher

Umm Al-Qura university, Makkah, Saudi Arabia.

Abstract: *SCRIB* gene is a member of planar cell polarity (PCP) genes which are involved in the process of neural tube closure (1). Homozygous *Scrib* mutations in mice cause craniorachischisis, the most severe typeof NTD. In humans, *SCRIB* mutations are associated with craniorachischisis (2). Recently, five deleterious mutations were identified in five infants with spins bifida, indicating that heterozygous *SCRIB* mutations may underlie thepathogenesis of human spina bifida (3).Here, we describe a 29 year- old male presenting with primary infertility due to non-obstructive azoospermia. In addition, he is suffering from urinary and fecal incontinence and club foot due to sacral myelomeningocele which was operated during the first weeks of his life. WES revealed de novo heterozygous variant p.(Arg277Trp) in *SCRIB* gene. *In silico* analysis imply potentially deleterious effect of this change. Mutations in SCRIB gene have been reported in few infants with variable NTDs. To our knowledge, this is the first case to be reported in adult patient. SCRIB gene role in spermatogenic failure has not been reported. Additional studies are required to evaluate its role in male infertility.

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Z.A. Azher: None.

P11.113.C SCUBE3 loss-of-function causes a recognizable developmental disorder due to defective bone morphogenetic protein (BMP) signaling

Marcello Niceta[1], *Yuh-Charn Lin*[2,3], *Valentina Muto*[1], *Barbara Vona*[4,5], *Alistair T. Pagnamenta*[6], *Reza Maroofian*[7], *Christian Beetz*[8], *Hermine van Duyvenvoorde*[9], *Maria Lisa Dentici*[1], *Peter Lauffer*[10], *Sadeq Vallian*[11], *Andrea Ciolfi*[1], *Simone Pizzi*[1], *Peter Bauer*[8], *Nana-Maria Grüning*[8], *Emanuele Bellacchio*[1], *Andrea Del Fattore*[1], *Stefania Petrini*[12], *Ranad Shaheen*[13,14], *Dov Tiosano*[15,16], *Rana Halloun*[15], *Ben Pode-Shakked*[17,18], *Hatice Mutlu Albayrak*[19], *Emregül Işık*[20], *Jan M. Wit*[20], *Marcus Dittrich*[4,21], *Bruna L. Freire*[22], *Debora R. Bertola*[23], *Alexander A. L. Jorge*[22], *Ortal Barel*[24], *Ataf H. Sabir*[25,26], *Amal M. Al Teneiji*[27], *Sulaima M. Taji*[27], *Nouriya Al-Sannaa*[28], *Hind Al-Abdulwahed*[28], *Maria Cristina Digilio*[1], *Melita Irving*[25], *Yair Anikster*[17,18,29], *Gandham SL Bhavani*[30], *Katta M. Girisha*[30], *Thomas Haaf*[4], *Jenny C. Taylor*[6], *Bruno Dallapiccola*[1], *Fowzan S. Alkuraya*[13], *Ruey-Bing Yang*[2,31,32], *Marco Tartaglia*[1]

[1]Genetics and Rare Diseases Research Division, IRCCS, Ospedale Pediatrico Bambino Gesù, Rome, Italy, [2]Department of Physiology, School of Medicine, Taipei Medical University, Taipei, Taiwan, [3]Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, [4]Institute of Human Genetics, Julius Maximilians University, Würzburg, Germany, [5]Department of Otolaryngology - Head and Neck Surgery, Eberhard Karls University, Tübingen, Germany, [6]NIHR Oxford Biomedical Research Centre, Wellcome Centre for Human Genetics, University of Oxford, Oxford, United Kingdom, [7]Genetics and Molecular Cell Sciences Research Centre, St George's University of London, Cranmer Terrace, London, United Kingdom, [8]Centogene AG, Rostock, Germany, [9]Department of Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands, [10]Department of Paediatric Endocrinology, Emma Children's Hospital, Amsterdam University Medical Center, Amsterdam, Netherlands, [11]Department of Cell and Molecular Biology & Microbiology, University of Isfahan, Isfahan, Iran, Islamic Republic of, [12]Confocal Microscopy Core Facility, Research Laboratories, IRCCS Ospedale Pediatrico Bambino Gesù, Rome, Italy, [13]Department of Genetics, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia, [14]Qatar Biomedical Research Institute, Hamad Bin Khalifa University, Doha, Qatar, [15]Pediatric Endocrinology Unit, Ruth Rappaport Children's Hospital, Rambam Healthcare Campus, Haifa, Israel, [16]Ruth and Bruce Rappaport Faculty of Medicine, Technion, Israel Institute of Technology, Haifa, Israel, [17]Edmond and Lily Safra Children's Hospital, Sheba Medical Center, Tel-Hashomer, Israel, [18]The Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel, [19]Department of Pediatric Endocrinology, Gaziantep Cengiz Gökcek Maternity & Children's Hospital, Gaziantep, Turkey, [20]Department of Pediatrics, Leiden University Medical Center, Leiden, Netherlands, [21]Institute of Bioinformatics, Julius Maximilians University, Würzburg, Germany, [22]Unidade de Endocrinologia Genética, Hospital das Clínicas da Faculdade de Medicina da Universidade de Sao Paulo, Sao Paulo, Brazil, [23]Unidade de Genética do Instituto da Criança, Hospital das Clínicas da Faculdade de Medicina da Universidade de Sao Paulo, Sao Paulo, Brazil, [24]Sheba Cancer Research Center, Sheba Medical Center, Tel-Hashomer, Israel, [25]Department of Clinical Genetics, Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom, [26]Birmingham Women's and Children's NHS Foundation Trust, University of Birmingham, Birmingham, United Kingdom, [27]Department of Paediatrics, Sheikh Khalifa Medical City, Abu Dhabi, United Arab Emirates, [28]Johns Hopkins Aramco Healthcare, Dhahran, Saudi Arabia, [29]Wohl Institute for Translational Medicine, Sheba Medical Center, Tel-Hashomer, Israel, [30]Department of Medical Genetics, Kasturba Medical College, Manipal, India, [31]Ph.D. Program in Drug Discovery and Development Industry, College of Pharmacy, Taipei Medical University, Taipei, Taiwan, [32]Institute of Pharmacology, School of Medicine, National Yang-Ming University, Taipei, Taiwan.

In the extracellular microenvironment, auxiliary proteins control cell behavior and coordinate embryo development by acting as co-receptors or direct antagonists of defective bone morphogenetic protein (BMP), Activin and TGF-β ligands. Pathogenic variants in genes encoding these proteins can dramatically affect development and physiology. Signal peptide-CUB-EGF domain-containing protein 3 (SCUBE3) is a member of a small family of multifunctional secreted or cell surface-anchored glycoproteins functioning as co-receptors for a variety of growth factors. Here we report that biallelic inactivating variants in *SCUBE3* have pleiotropic consequences on development and cause a previously unrecognized syndromic disorder. Eighteen affected individuals from nine unrelated families showed a consistent phenotype characterized by re growth restriction, skeletal defects, distinctive craniofacial appearance, and dental anomalies. In vitro functional validation studies demonstrated a variable impact of disease-causing variants on transcript processing, protein secretion and function, and their dysregulating effect BMP signaling. We show that SCUBE3 is an auxiliary protein that acts as a BMP2/BMP4 co-receptor, recruits the BMP receptor complexes into raft microdomains, and positively modulates signaling possibly by augmenting the specific interactions between BMPs and BMP type I receptors. *Scube3*$^{-/-}$ mice showed craniofacial and dental defects, reduced body size and defective endochondral bone growth due to impaired BMP-mediated chondrogenesis and osteogenesis, recapitulating the human disorder. Our findings identify the first human disease caused by defective function of a member of the SCUBE family, and link SCUBE3 to processes controlling growth, morphogenesis, and bone and teeth development through modulation of BMP signaling.
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P11.114.D Genetic testing algorithms for fetal malformations malformations

Viorica Radoi[1], *Radu-Ioan Ursu*[1], *Cristina Dragomir*[2], *Andreea Ionescu*[2], *Iuliana Chelu*[2], *Laurentiu Camil Bohiltea*[1]

[1]INSMC Alessandrescu-Rusescu, Bucharest, Romania, [2]Synevo Romania, Bucharest, Romania.

Fetal abnormalities are diagnosed in 3-5% of all pregnancies. In the case of perinatal death, congenital anomalies occur in 20-25% of the patients. The prenatal genetic tests recommended are QF-PCR for rapid aneuploidy testing, the G-banded karyotyping and array comparative genomic hybridization (aCGH) for the detection of chromosomal anomalies and CNVs. QF-PCR identifies trisomies in around 30% of dysmorphic fetuses. Karyotyping detects pathogenic chromosomal rearrangements in a further 5%. aCGH finds additional pathogenic CNVs in 3% to 6.5% of structurally abnormal fetuses with normal karyotypes. Using these tests in combination, an underlying genetic aetiology can be identified in up to 40% of dysmorphic fetuses, still leaving the majority of cases undiagnosed.Whole exome sequencing (WES) offers the chance of identifying the final genetic diagnosis, accurate estimation of recurrence risks, but is recommended in specific selected situations and comes with certain disadvantages.Case reportPatient: 27 years old, 23 weeks pregnancy,with negative medical family history.Ultrasound revealed strawberry shaped head, mid-face hypoplasia Amniocentesis: fetal karyotype and aCGH – normal resultFetal DNA testing by WES identified TWIST1 gene with variant c.82C>T (p.Gln28Ter) mutation - pathogenic - Saethre Chotzen syndromeThe advances of genomic medicine are impacting prenatal diagnosis, just like any other medical field. While these innovations offer exciting new opportunities and can empower families with increased knowledge about their reproductive risks and with decision-making autonomy, they have to be carefully introduced in an evidence-based and ethically responsible manner and monitored after implementation.

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P11.115.A Diagnostic utility of next-generation sequencing panel tests in the diagnosis of skeletal dysplasias

*Tiia Kangas-Kontio[1], Alicia Scocchia[2], **Satu Valo**[1], Kimberly Gall[2], Liisa Pelttari[1], Johanna Huusko[1], Jonna Tallila[1], Inka Saarinen[1], Johanna Sistonen[1], Juha Koskenvuo[1], Tero-Pekka Alastalo[2]*

[1]Blueprint Genetics, Espoo, Finland, [2]Blueprint Genetics Inc, a Quest Diagnostics Company, Seattle, WA, USA.

Introduction: Skeletal dysplasias encompass over 450 heritable conditions with significant phenotype overlap. In this study, we retrospectively assessed the diagnostic utility of next-generation sequencing (NGS) panel tests containing genes associated with skeletal dysplasias and growth disorders.

Materials and Methods: Clinical reports of 543 patients who underwent panel testing at Blueprint Genetics with an indication of suspected skeletal dysplasia or growth disorder were examined. The patients received one of three nested panel tests containing 113, 251 and 374 genes, respectively. Panel testing included both sequence and copy number variant (CNV) analyses of NGS data from a clinically validated exome assay .

Results: A molecular diagnosis was established in 42.0% of patients. Diagnostic yield was significantly higher among fetal samples (62.5%, n = 55/88) compared to post-natal samples (38%, n = 173/455; z = 4.2489, $P < 0.00001$). Twelve diagnostic CNVs were reported, ranging in size from 241 bp to 6.7 Mb, representing 5.3% of diagnostic findings. Five diagnostic CNVs involved loss of the *SHOX* gene in the pseudoautosomal region. Diagnostic variants were identified in 71 genes, with nearly half of these genes (n = 33, 46.5%) contributing uniquely to a molecular diagnosis for a single patient.

Conclusions: This study demonstrates the utility of panel testing for patients with a suspected skeletal dysplasia or growth disorder, with diagnostic yield of 42%. Additionally, the high diagnostic yield seen in prenatal cases is valuable information for genetic counseling regarding natural history and prognosis. Pursuing comprehensive panel testing with high-resolution CNV analysis can provide a time-sensitive diagnostic benefit, given the considerable phenotype overlap amongst skeletal dysplasias.

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P11.116.B Novel *SMARCA4* mutation identified in a patient with a mild phenotype of Coffin-Siris syndrome

Beata Aleksiuniene[1,2], *Loreta Cimbalistiene[1,2], Algirdas Utkus[1]*

[1]Department of Human and Medical Genetics, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, Vilnius, Lithuania, [2]Centre for Medical Genetics, Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania.

Coffin-Siris syndrome (CSS) is a rare syndrome characterized by developmental delay, craniofacial abnormalities, hypoplastic or absent fifth digits/nails of the hands and feet and other variable clinical manifestations. CSS is caused by mutations in several genes encoding components of the BAF complex. It has been demonstrated that CSS clinical manifestation may vary, depending on the variants in the particular gene. We report on a 7-years-old female referred for evaluation because of mild dysmorphic features and congenital heart disease - supravalvular aortic stenosis. She was born in a normal delivery but with lower birth-weight (2850 g). Psychomotor developmental delay was observed since infancy, and then she was diagnosed with mixed developmental disorder, learning difficulties. The following dysmorphic features were noted: facial coarseness was minimal - thick eyebrows, mild micrognatia, long broad philtrum, nose with bulbous tip and anteverted nostrils. Hypoplasia of bilateral fifth toes as well as hypoaplasia of bilateral fifth toenails were significant. Whole exome sequencing analysis of patient's DNA revealed the novel heterozygous nonsense variant c.646C>T, p.(Gln216Ter) in the *SMARCA4* gene. The variant was confirmed by Sanger sequencing. This alteration causes a cytosine to thymine transition, resulting a premature termination codon in the *SMARCA4* protein. According to *in silico* analysis, the identified c.646C>T variant is classified as pathogenic. Our report futher supports the notion that nonsense variants of *SMARCA4* cause mild delay phenotype of CSS. However, further studies are needed in order to better characterize the phenotype and establish genotype-phenotype correlation.

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P11.117.C SNP array analysis as a detection tool for single gene disorders

Elanur Yilmaz[1,2], *Esra Borklu[1], Umut Altunoglu[1], Sahin Avci[1], Serpil Eraslan[1], Hulya Kayserili[1]*

[1]Medical Genetics Department, Koç University School of Medicine (KUSOM), ISTANBUL, Turkey, [2]Koç University Research Center for Translational Medicine (KUTTAM), Istanbul, Turkey.

Single Nucleotide Polymorphism array(SNP-a) provides an efficient,powerful, and high-throughput analysis for structural chromosomal variations,microdeletions and microduplications. Furthermore,single-gene disorders can also successfully be detected by SNP-a. SNP-a was performed on 37 postnatal cases in 2020 to identify the genetic etiopathogenesis of congenital/neurodevelopmental anomalies by Illumina CytoSNP v12. Pathogenic/possibly pathogenic variations were found in 21. Four microdeletions were considered suggestive for well-delineated single-gene syndromes, namely Nance-Horan(NHS),Phelan-McDermid(PMS),Hand-Foot-Genital(HFGS),and Feingold Type2(FS2).The first microdeletion,harbored the *NHS* along with 21 other genes in Xp22.2p22.13(16051468_18313707)(GRCh37/hg19).The boy had microphthalmia/cataract,mild global developmental delay,ventricular septal defect which was well overlapped with the clinical findings of NHS. The second microdeletion,7.17Mb,encompassed 51 OMIM-genes including the *SHANK3* in 22q13.2q13.33 (43996288_51169045)(GRCh37/hg19).The 22q13 microdeletion or mutations of the *SHANK3* are associated with psychomotor and speech delay.Accompanying features of the case excluded for Beckwith-Wiedemann Syndrome were large for gestational age, macroglossia,renal anomalies, asymmetric growth could due to

the impact of other deleted genes.The third case displayed 0.3Mb deletion in 7p15.2(26,938,809_27,262,849)(GRCh37/hg19) harboring the *HOXA13*. Skeletal findings,borderline intellectual disability, vesicoureteral reflux of the case could be explained by HFGS. The fourth, 4.17Mb deletion harbored nine OMIM-genes including *MIR17HG* in 13q31.3(90513153_94681840)(GRCh37/hg19).The phenotypic features like microcephaly,brachydactylic fingers and toes,dysmorphisms,borderline intellectual disability of the girl was in correlation with FS2 diagnosis.

Conclusion: Chromosomal microdeletions/microduplications are one of the key classes of variations that could reveal the etiopathogenesis of single-gene disorders.In the case of non-specific,or missing pivotal clinical findings for a syndrome, following an algorythmic diagnostic approach and performing the SNP-a can pinpoint a single-gene disorders and together with the reverse phenotyping,the clinical diagnosis can also be determined.

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P11.119.A Beyond the known phenotype of Sotos Syndrome: Spanish cohort of 31 pediatric patients

Antonio F. Martinez-Monseny[1], *Lourdes R. Vega-Hanna*[1], *Mario Sanz-Cuesta*[2], *Didac Casas-Alba*[1], *Mercè Bolasell*[1], *Loreto Martorell*[1], *Leticia Pias*[1], *Mercedes Serrano*[1]

[1]*Sant Joan de Deu Hospital, Barcelona, Spain,* [2]*Hospital de Sant Boi, Parc Sanitari Sant Joan de Déu, Barcelona, Spain.*

Introduction: Sotos Syndrome (SS, OMIM#117550) is a heterogeneous genetic condition, recognized by overgrowth, macrocephaly, typical facial appearance and intellectual disability. SS can be classified in three different subtypes according to molecular findings in NSD1, NFIX and APC2 genes. We aim to expand the phenotype studying a cohort of patients, describing their expected and unexpected clinical features.

Methods: descriptive and retrospective study including genetically confirmed SS subjects, followed at a tertiary pediatric hospital from June 2019 to December 2020.

Results: Thirty-one patients (16 males) with SS were included, 27 with NSD1 variants and 4 with NFIX variants, bearing predominantly point mutations in both cases. Seven individuals were born prematurely. All individuals presented with overgrowth, typical dysmorphic features and different degrees of intellectual disability. Although structural cardiac defects are frequent, here we describe a statistically significant concomitant presentation with hypotonia ($p = 0.04$), as well as non-structural diseases (pericarditis and arrhythmia) that were outstanding in our cohort. Epilepsy was observed in 14 (45.2%) and scoliosis in 11 (40.7%). We describe novel oncological malignancies not previously linked to SS such as splenic hamartoma, retinal melanocytoma, acute lymphocytic leukemia and prenatal neuroblastoma, which were more prevalent in patients with missense mutations ($p = 0.04$). Five patients suffered from recurrent onychocryptosis that required surgical procedures, as an unreported medical condition. Interestingly, this finding was more prevalent in patients with macrocephaly ($p = 0.04$).

Conclusions: Our study focuses on undescribed features in SS, expanding the clinical and molecular spectrum and advising clinicians about some unreported clinical complications. We suggest novel genotype-phenotype correlations.

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P11.120.B Two different syndromic craniosynostosis in the same family

Semra Gürsoy[1], *Filiz Hazan*[2]

[1]*Department of Pediatric Genetics, Dr. Behcet Uz Children's Hospital, Izmir, Turkey,* [2]*Department of Medical Genetics, Dr. Behcet Uz Children's Hospital, Izmir, Turkey.*

Introduction: Craniosynostosis is characterized by the premature fusion of calvarial sutures and can be isolated without any additional anomalies, or as a part of a syndrome. Syndromic craniosynostosis with a certain genetic cause is more likely to involve multiple sutures or bilateral coronal sutures. *FGFR2*, *FGFR3*, *FGFR1*, *TWIST1* and *EFNB1* are major causative genes of genetic syndromes associated with craniosynostosis. Herein, we present two different syndromic craniosynostosis in the same family.

Material and Methods: *TWIST1* and *EFNB1* genes were sequenced by using Sanger sequencing.

Results: A 7-month-old female patient was referred to our genetic clinic for dysmorphic features including hypertelorism, broad forehead, down-slanting palpebral fissures and brachycephaly, compatible with Saethre-Chotzen syndrome. Bilateral coronal synostosis was detected on three dimensional cranial computed tomography. *TWIST1* gene was sequenced and a nonsense mutation (c.301C>T; p.Q101X) was revealed. The father of the patient who had similar phenotypic features, also had the same mutation. Additionally, the index patient had a 4,5 year-old sister whose facial features were compatible with Craniofrontonasal syndrome. A heterozygous nonsense mutation of *EFNB1* gene (c.196C>T; p.R66X) was detected in this patient. The mother had also unilateral coronal craniosynostosis, unilateral proptosis and unilateral ptosis, longitudinal splitting of nails and clinodactyly. The same *EFNB1* gene mutation was detected in the mother.

Conclusions: The detailed clinical evaluation is crucial for the correct diagnosis of genetic syndromes associated with craniosynostosis due to its phenotypical variability. Furthermore, molecular diagnosis may have an important role for genetic counceling and prediction of the prognosis.

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P11.121.C Analysis of exome data of a nationally identified cohort of 603 patients with syndromic orofacial clefting

Kate Wilson[1], *Dianne Newbury*[2], *Usha Kini*[1,3]

[1]*Oxford Centre for Genomic Medicine, Oxford University Hospitals NHS Foundation Trust, Oxford, United Kingdom,* [2]*Faculty of Health and Life Sciences, Oxford Brookes University, Oxford, United Kingdom,* [3]*Spires Cleft Centre, John Radcliffe Hospital, Oxford, United Kingdom.*

Introduction: Syndromic orofacial clefting (OC) accounts for 30% of cleft lip and/or palate. We reviewed variants identified by exon-arrayCGH and exome sequencing (ES) in patients with syndromic OC within the Deciphering Developmental Disorders (DDD) study to investigate molecular pathways associated with syndromic OC.

Materials and Methods: Patients with HPO terms containing 'cleft' and 'bifid uvula' were identified through a complementary analysis project within the DDD study. Possible diagnostic variants were identified by automated variant filtering and manual review and deposited into DECIPHER. Single nucleotide variants within known disease-causing genes and copy number variants were classified according to ACMG guidelines, the ACGS Best Practice Guidelines and consensus opinion. Molecular pathway analyses

were performed within STRING to investigate patterns of gene function in syndromic OC and across OC types.

Results: 603/13612 (4.4%) patients were identified of whom 453/603 (75.1%) had trio ES. 448/603 (74.3%) patients had cleft palate, 132/603 (21.9%) had cleft lip +/- palate and 23/603 (3.8%) had oral clefting. 259/603 (43%) patients had moderate to profound intellectual disability and/or global developmental delay. Likely pathogenic or pathogenic variants were identified for 220/603 (36.5%) patients in 124 known disease-causing genes with *SATB2* being the most common (16/220, 7.3%). Gene ontology and pathway analyses suggested that the molecular mechanisms underlying syndromic OC were distinct from those in non-syndromic OC.

Conclusions: Using an ES approach in a large cohort of patients with syndromic OC we identified molecular pathways and several new genes that are not traditionally known to be associated with clefting.

K. Wilson: None. **D. Newbury:** None. **U. Kini:** None.

P11.122.D *TAB2* deletions and loss-of-function variants cause a Noonan-like syndrome with mitral valve disease, cardiomyopathy and hypermobility

Aafke Engwerda[1], *Erika K. S. M. Leenders*[2], *Barbara Frentz*[3], *Paulien A. Terhal*[4], *Katharina Löhner*[1], *Bert B. A. de Vries*[2], *Trijnie Djikhuizen*[1], *Yvonne J. Vos*[1], *Tuula Rinne*[2], *Maarten P. van den Berg*[5], *Marc T. R. Roofthooft*[6], *Patrick Deelen*[1,4], *Conny M. A. van Ravenswaaij-Arts*[1], *Wilhelmina S. Kerstjens-Frederikse*[1]

[1]*University of Groningen, University Medical Center Groningen, Department of Genetics, Groningen, Netherlands,* [2]*Department of Human Genetics, Radboud University Medical Center, Nijmegen, Netherlands,* [3]*Vanboeijen, Assen, Netherlands,* [4]*Department of Genetics, Utrecht University Medical Center, Utrecht, Netherlands,* [5]*University of Groningen, University Medical Center Groningen, Department of Cardiology, Groningen, Netherlands,* [6]*University of Groningen, University Medical Center Groningen, Beatrix Children's Hospital, Department of Paediatric Cardiology, Groningen, Netherlands.*

Introduction: *TAB2* loss-of-function variants and deletions including *TAB2* are associated with congenital heart defects and cardiomyopathy. In literature occasionally other features have been mentioned, including, short stature, facial dysmorphisms, connective tissue abnormalities and a variable degree of developmental delay. However, these features have not been linked to *TAB2* thus far. We aimed to confirm that the phenotype in 6q25.1 deletion patients is caused by haploinsufficiency of *TAB2*.

Materials and Methods: Within the Chromosome 6 Project, a large social media–based study, we observed a shared phenotype among 6q25.1 deletion patients. We identified our candidate gene *TAB2* and subsequently sequenced *TAB2* in patients with matching phenotypes. We also recruited patients with pathogenic *TAB2* variants detected by exome sequencing. Clinical data were compared with literature cases.

Results: We identified 11 patients with a deletion containing *TAB2* (size 1.68-14.31 Mb) and 14 patients from six families with novel truncating *TAB2* variants. Twenty (80%) patients had cardiac diseases, often mitral valve defects and/or cardiomyopathy. Eighteen (72%) had short stature and 18 (72%) had hypermobility. Twenty patients (80%) had facial features suggestive for Noonan syndrome. No substantial phenotypic differences were noted between patients with deletions and those with intragenic variants. In comparison, all 45 patients from literature had cardiac diseases but syndromic features were reported infrequently.

Conclusions: This study shows that the 6q25.1 deletion phenotype is caused by haploinsufficiency of *TAB2* and that *TAB2* is not just associated with cardiac disease, but also with a distinct Noonan-like phenotype. We propose the name "*TAB2*-related syndrome".

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P11.123.A Tenorio syndrome: description of 9 new cases and review of the clinical and molecular features

Jair Tenorio[1], *Pedro Arias*[1], *Alberto Fernandez-Jaen*[2], *Guillermo Lay-Son*[3], *Allan Bayat*[4], *Laurence Olivier-Faivre*[5], *Natalia Gallego*[1], *Sergio Ramos*[1], *James Lespinasse*[6], *Frederic Tran-Mau-Them*[7], *Fernando Santos-Simarro*[1], *Lucile Pinson*[8], *Antonio Federico Martinez-Monseny*[9], *María del Mar O´Callaghan Cord*[9], *Pablo Lapunzina*[1]

[1]*Institute of Medical and Molecular Genetics (INGEMM) - Hospital Universitario La Paz, Madrid, Spain,* [2]*Hospital Universitario Quirón de Madrid, Madrid, Spain,* [3]*Pontificia Universidad Catolica de Chile, Santiago de Chile, Chile,* [4]*Department of Pediatrics, Hvidovre Hospital. University of Copenhagen, Copenhagen, Denmark,* [5]*Hôpital d'enfants CHU Dijon Bourgogne - Hôpital François Mitterrand 2 bd Maréchal de Lattre de Tassigny 21000 Dijon, Dijon, France,* [6]*Service de Cytogenetique, Centre Hospitalier de Chambéry, Chambéry, France, Chambéry, France,* [7]*UF6254 Innovation en Diagnostic Genomique des Maladies Rares Bat B3, 15 boulevard du Maréchal de Lattre de Tassigny 21000 Dijon, France, Dijon, France,* [8]*Département de Génétique Médicale, Maladies Rares et Médecine Personnalisée, CHU de Montpellier, Montpellier, France, Montpellier, France,* [9]*Hospital Sant Joan de Déu, Barcelona, Spain, Barcelona, Spain.*

Tenorio syndrome (TNORS) is a relatively recent disorder with very few cases described so far. Clinical features include macrocephaly, intellectual disability, hypotonia, enlarged ventricles and autoimmune diseases. Molecular underlying mechanism included missense variants and large deletions encompassing RNF125, a gene that encodes for an U3 ubiquitin ligase protein, involved in the regulation of several proteins by its binding and degradation through the proteasome. Since the initial description of the disorder and families, several new patients were diagnosed, adding more evidence of the clinical manifestations. Thus, the aim of this project is to perform a deep phenotyping of the current cases and review all cases in which a pathogenic variant has been found in RNF125. Interestingly, not all patients with pathogenic variants in RNF125 manifest overgrowth, but instead, there is a common pattern of neurodevelopmental disease, with mild to moderate degrees. Segregation analysis showed that in some cases, though the variant was inherited by an apparently normal parent, deep phenotyping suggested a mild form of the disease in their progenitors. The mechanism underlying the development of this disease is not well understood yet and the description of more cases will help to a better understanding and clinical characterization. In summary, we report nine new cases of Tenorio syndrome (MIM, 616260), in patients with a variable degree of the disease, and a common concurrent neurodevelopmental disorder. Not all cases have overgrowth, and this must be considering for a correct diagnosis. Grants: FIS-PI20/01053

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P11.124.B Unravelling terminal 6p deletions with the help of social media

Eleana Rraku[1], *Aafke Engwerda*[1], *Jennifer Geurink*[1], *Laura Monsma*[1], *Pauline Bouman*[2], *Morris A. Swertz*[1], *Trijnie Dijkhuizen*[1], *Conny M. A. van Ravenswaaij-Arts*[1]

[1]*University of Groningen, University Medical Center Groningen, Department of Genetics, Groningen, Netherlands,* [2]*Chromosome 6 Facebook Group, Utrecht, Netherlands.*

Introduction: Chromosome 6 deletions are rare, and information on their clinical consequences is scarce. Parents of affected individuals often turn to the internet for information and support. The Chromosome 6 Project collaborates with parents to study the phenotypes of chromosome 6 aberrations. We hereby present our results on terminal 6p deletions.

Materials and Methods: Parents were notified of the study through social media and were requested to upload a microarray report. Phenotype data was collected directly from parents via a multilingual online questionnaire, which was also used for cases collected through a literature search. Four subgroups were created based on deletion sizes. The phenotypes of the total group and each subgroup were described.

Results: Twelve Chromosome 6 Project participants and 32 literature cases were included, making this the largest cohort of terminal 6p deletions. Deletion sizes ranged from 0.3 to 22.3 Mb (median 4.0 Mb). The total group was characterized by an Axenfeld-Rieger anomaly, vision problems, hearing impairment, hypotonia, dysmorphic features, cardiac and brain defects (cerebellar abnormalities and ventriculomegaly). Developmental delay was mostly mild. These traits were observed in all subgroups, suggesting a dominant role for the most distally deleted genes. One of these genes is *FOXC1*, known to cause Axenfeld-Rieger syndrome. In the subgroup with the largest deletions (>7.15 Mb), ventricular septal defects and kidney abnormalities were also observed.

Conclusions: The most distally located genes play a determining role in the phenotypes of terminal 6p deletions. Furthermore, we demonstrate the power of social media in studying rare diseases.

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P11.125.C *TNRC6A* is a candidate gene for a phenotype with multisystem involvement

Sanna Puusepp[1,2], *Tiia Reimand*[1,2], *Chris Pruunsild*[3], *Nina Powell-Hamilton*[4], *Kari Magnussen*[5], *George Anadiotis*[5], *Katrin Õunap*[1,2]

[1]*Department of Clinical Genetics, Institute of Clinical Medicine, Faculty of Medicine, University of Tartu, Tartu, Estonia,* [2]*Department of Clinical Genetics, United Laboratories, Tartu University Hospital, Tartu, Estonia,* [3]*Department of General Pediatrics and Neurology, Children's Clinic, Tartu University Hospital, Tartu, Estonia,* [4]*Division of Medical Genetics, Nemours/Alfred I. duPont Hospital for Children, Wilmington, DE, USA,* [5]*Randall Children's Hospital at Legacy Emanuel, Portland, OR, USA.*

Trinucleotide repeat-containing gene 6A (*TNRC6A*) encodes the GW182 protein, which is involved in miRNA induced gene silencing by assembling Argonaute proteins with target mRNA. Studies of the deficiency of GW182 in mice and its orthologue gawky in Drosophila demonstrated defects in embryonic development. Patients with GW182 autoantibodies showed neuropathy, ataxia, arthritis, rheumatologic diseases, and cancers. Recently, pathogenic heterozygous TTTCA repeat expansion in *TNRC6A* was identified in five related patients with benign adult familial myoclonic epilepsy. We report three unrelated male patients with loss-of-function variants in *TNRC6A* and partially overlapping phenotypes. Patient 1 presented with speech delay, autism, frequent headaches, balance problems, muscular hypotonia, mild proximal and facial muscle weakness, dysarthria, fatigue, scoliosis, connective tissue weakness, joint hypermobility, juvenile idiopathic arthritis, osteoporosis with compression fractures, arterial hypertension, and obesity. Notable findings for Patient 2 include neonatal stroke, developmental delay, seizures, patent foramen ovale, cervical ribs, unilateral auricular tag, and sacral dimple. Interestingly, these two patients share similar facial features with epicanthic folds and cupped ears. Patient 3 has a history of autism spectrum disorder, migraines, chronic fatigue, muscle weakness, and gastroesophageal reflux. Whole exome sequencing revealed different de novo heterozygous variants in *TNRC6A*: c.4405C>T, p.(Q1469X); c.3474dupA, p.(E1159RfsX3) and c.2570G>A, p.(W857X). All variants are absent from the gnomAD database. In conclusion, we are highly suspicious that the de novo variants in the candidate gene *TNRC6A* are clinically relevant, however, additional cases with overlapping findings and functional studies are needed to reach more solid conclusions. Funding: Estonian Research Council grant PRG471

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P11.126.D Unravelling the effects of germline missense variants in TRAF7

Aina Prat-Planas[1], *Laura Castilla-Vallmanya*[1], *Miguel Subías*[1], *Mónica Centeno-Pla*[1], *Daniel Grinberg*[1], *Raquel Rabionet*[1], *Roser Urreizti*[1,2], *Susanna Balcells*[1]

[1]*Department of Genetics, Microbiology and Statistics, Faculty of Biology, University of Barcelona, IBUB, IRSJD, CIBERER, 08028, Barcelona, Spain,* [2]*Clinical Biochemistry Department, Hospital Sant Joan de Déu, CIBERER, 08950, Esplugues de Llobregat, Spain.*

Introduction: *TRAF7* codes for a protein that acts as E3 ubiquitin ligase in several signalling pathways mediated by Tumour Necrosis Factor (TNF) family ligands. Somatic mutations in *TRAF7* have been associated with tumorigenic processes, while germline mutations have been described as disease-causing for the TRAF7 syndrome, an ultra-rare disorder characterized by intellectual disability, motor delay, cardiac alterations and dysmorphic features. Our aim was to explore the biological effects of germline missense variants in *TRAF7*.

Material and Methods: We performed an mRNA-Seq analysis in skin fibroblasts from 3 TRAF7 syndrome patients and 6 controls, with and without TNF-α stimulation. Results were subjected to differential expression, pathway enrichment analysis and qPCR validation. Additionally, we used a siRNA strategy to knock-down *TRAF7* in control fibroblasts and analysed the expression of selected genes by qPCR.

Results: We identified 78 differentially expressed genes between patients and controls, 8 of which successfully validated by qPCR. Enrichment analysis highlighted several pathways that are associated with the most relevant phenotypes of the syndrome (Genet Med 2020; 22:1215-1226). The analysis of the expression of candidate genes in TRAF7-KD fibroblasts showed promising results for *ANGPT1* and *CASK*, found downregulated in patients but significantly upregulated in TRAF7-silenced cells.

Conclusions: Gene expression alterations caused by germline missense variants in *TRAF7* partly explain the phenotype observed in patients. TRAF7 knockdown data suggest that these variants cause a gain-of-function effect in some of TRAF7 functions.

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P11.127.A Array CGH confirmation of a de novo reciprocal translocation involving X and 16 chromosomes

*Nouha Bouayed ABDELMOULA, **Balkiss Abdelmoula**, Sonda Kammoun, Saloua Ben Amor, Souad Kammoun*

Genomics of signalopathies at the service of medicine UR17ES36, Medical University of Sfax, Sfax, Tunisia.

Objective: Array comparative genomic hybridization (CGH) is nowadays the best tool to identify chromosomal abnormalities. The aim of this study was to identify a de novo reciprocal translocation.

Material and Methods: A Tunisian 13-day-old male newborn was referred to our genetic counselling for complex congenital heart disease (CHD) combining tetralogy of Fallot and pulmonary arteries hypoplasia. Conventional and molecular cytogenetic analysis were carried out to delineate the genetic aetiology of his syndromic CHD. Array-CGH was conducted using optimized DNA preparations and constitutional chip 4.0 microarray from PerkinElmer, USA.

Results: the newborn had dysmorphic features with dolichocephaly, micro-retro-gnathism, narrow/high-arched palate, low-set malformed ears, long philtrum, thin superior lip, upturned nares and periorbital fullness. Further findings were right hydrocele testis, and a small sacral dimple. Karyotyping revealed a de novo unbalanced translocation: 46,XY,der(16) t(?;16). The additional material in the distal extremity of the chromosome 16 was difficult to identify by RHG banding. Array-CGH analysis identified the Xp chromosome origin of the additional chromosomal material translocated to the subtelomeric region of chromosome 16q. It was therefore possible to reveal an Xp duplication but without detection of a distal 16q monosomy. However, breakpoints mapping and eventual deletion of the distal part of chromosome 16q were not possible. Although partial X duplication, the patient had no genital ambiguity or psychomotor disabilities until 7 months of age.

Conclusion: Our patient may have a KBG (MIM 148050) overlapping phenotype.

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P11.129.C Looking beyond mitral valve prolapse and ischaemic stroke - a late diagnosis of trichorhinophalangeal syndrome type I

***Marta P. Soares**[1], Márcia Rodrigues[1], Daniela P. Silva[2], Teresa Pinho Melo[2], Ana Berta Sousa[1]*

[1]Serviço de Genética Médica, Hospital de Santa Maria, Centro Hospitalar Universitário Lisboa Norte, Lisboa, Portugal, [2]Serviço de Neurologia, Departamento de Neurociências e Saúde Mental, Hospital de Santa Maria, Centro Hospitalar Universitário Lisboa Norte, Lisboa, Portugal.

Introduction: Trichorhinophalangeal syndrome type I (TRPS1) is a rare genetic disorder characterized by distinctive facial features (bulbous pear-shaped nose), ectodermal anomalies (sparse hair, dental anomalies, dystrophic nails), and skeletal findings (brachydactyly, cone-shaped epiphyses of phalanges, short stature). Less frequently (15%), variable cardiac abnormalities have been described, including mitral valve prolapse (MVP), reported in only seven patients.

Case report: A 36 year-old man was referred to Genetics due to facial dysmorphism, MVP and stroke at a young age. He was the second child of non-consanguineous parents with no relevant family history. Pregnancy and birth were uneventful; growth and psychomotor development were normal. At age 5, he was diagnosed and treated for unilateral Perthes disease. He evolved with arterial hypertension, and severe mitral regurgitation with MVP diagnosed at 30 years, requiring mitral valve plasty and anticoagulation therapy for two years. At 36 years, he had an ischaemic stroke, causing left-sided hemiparesis, treated with t-PA. After young stroke investigation, cardioembolic aetiology was considered and anticoagulation was reinitiated. Physical examination revealed short stature, sparse scalp hair, macrocephaly, facial dysmorphism (down-slanting palpebral fissures, peculiar nose with broad ridge and tip and underdeveloped alae, and low-set ears), and brachydactyly.

Results: Skeletal survey showed brachydactyly and cone-shaped epiphyses of phalanges, corroborating the clinical suspicion of TRPS1. A custom skeletal dysplasia NGS panel identified a heterozygous pathogenic variant in *TRPS1*: c.2831G>T, p.(Arg944-Met), confirming TRPS1.

Conclusion: This case illustrates a rare cardiac anomaly with impact on prognosis, warranting systematic screening for MVP in the management of TRPS1.

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P11.130.D Epileptic encephalopathy as a new feature of TSPYL1 variants, associated with sudden infant death with dysgenesis of the testes

***Benoit Mazel**[1,2,3], Candace Ben Signor[4], Véronique Darmency[5], Delphine Mallet[6], Valentin Bourgeois[7], Margot Grisval[1], Alexandra Pillard[3], Charlotte Poe[7], Marie Bournez[1], Christel Thauvin-Robinet[1,2,7], Antonio Vitobello[3,7], Yannis Duffourd[3,7], Christophe Philippe[3,7], Laurence Faivre[1,2,7], Sophie Nambot[1,2,7]*

[1]Centre de Génétique et Centre de Référence Anomalies du Développement et Syndromes Malformatifs, FHU TRANSLAD - Centre Hospitalier Universitaire Dijon Bourgogne, Dijon, France, [2]Fédération Hospitalo-Universitaire Médecine Translationnelle et Anomalies du Développement (FHU TRANSLAD), Centre Hospitalier Universitaire Dijon Bourgogne et Université de Bourgogne-Franche Comté, Dijon, France, [3]Laboratoire de Génétique chromosomique et moléculaire, UF Innovation en diagnostic génomique des maladies rares, Centre Hospitalier Universitaire Dijon Bourgogne, Dijon, France, [4]Centre de référence du syndrome de Prader-Willi et autres syndromes avec

troubles du comportement alimentaire, Centre Hospitalier Universitaire Dijon Bourgogne, Dijon, France, [5]Service de Neurophysiologie Clinique, Hôpital d'Enfants, Centre Hospitalier Universitaire Dijon Bourgogne, Dijon, France, [6]Service d'hormonologie, endocrinologie moléculaire et maladies rares, CPBE, groupement hospitalier Lyon-Est, Lyon-Bron, France, [7]Inserm UMR1231 GAD, Génétique des Anomalies du Développement, Université de Bourgogne, Dijon, France.

Introduction: Sudden infant death with dysgenesis of the testes syndrome (SIDDT) is a rare autosomal recessive disorder associating developmental sex disorder (DSD) in patients with 46,XY karyotype and visceroautonomic dysfunction responsible for sudden death before twelve months of age. It has been first described in 2004 and very few patients were since reported. We describe here a new patient issued from non-consanguineous parents with SIDDT and epileptic encephalopathy.

Methods: We provide the phenotypic description and genetic results of the first case carrying compound heterozygous *TSPYL1* variants. We also reviewed the data of the 21 initially described and the 5 recently reported patients with SIDDT.

Results: All literature's cases carried homozygous variants in *TSPYL1* and were issued from consanguineous parents. All 27 patients presented with sudden infant death and all patients with a 46,XY karyotype had DSD. Our patient presented at the age of 4.5 months with repeated seizures. Within a month, his neurological status deteriorated leading to a severe intractable epileptic encephalopathy. He died at age ten months of cardiorespiratory arrest. Four other reported patients among two families presented with progressive epilepsy, including one with severe epileptic encephalopathy. They died between five and nine months. No similar phenotype was described in the 22 other patients.

Conclusions: These findings expand the phenotypic spectrum of SIDDT, by reporting progressive epilepsy and severe epileptic encephalopathy as a possible outcome. This information may help in managing patients with SIDDT.

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P11.131.A X-linked variants in *SHROOM4* are implicated in the formation of VACTERL

Caroline M. Kolvenbach[1,2,3], Tim Felger[2], Luca Schierbaum[1,3], Isabelle Thiffault[4], Thomas Smol[5], Allan Bayat[6], Frederic Thieme[3], Kerstin U. Ludwig[3], Öznur Yilmaz[2], Tobias Lindenberg[2], Ute Moog[7], Alina C. Hilger[1,3], Phillip Grote[8], Benjamin Odermatt[2], Heiko Reutter[3,9], ***Gabriel C. Dworschak***[1,2,3]

[1]*Department of Pediatrics, University Hospital Bonn, Bonn, Germany,* [2]*Institute of Anatomy, University of Bonn, Bonn, Germany,* [3]*Institute of Human Genetics, Medical Faculty, University of Bonn, Bonn, Germany,* [4]*Center for Pediatric Genomic Medicine, Children's Mercy Hospital, Kansas City, KS, USA,* [5]*Institut de Génétique Médicale, Hopital Jeanne de Flandre, Lille University Hospital, Lille, France,* [6]*Department of Genetics and Personalized Medicine, Danish Epilepsy Centre, Dianalund, Denmark,* [7]*Institute of Human Genetics, University of Heidelberg, Heidelberg, Germany,* [8]*Institute of Cardiovascular Regeneration, Center for Molecular Medicine, Goethe University, Frankfurt am Main, Germany,* [9]*Department of Neonatology and Pediatric Intensive Care, University Hospital Bonn, Bonn, Germany.*

Introduction: The acronym VATER/VACTERL association refers to the non-random co-occurrence of the following component features: vertebral defects (V), anorectal malformations (A), cardiac defects (C), tracheoesophageal fistula with or without esophageal atresia (TE), renal malformations (R), and limb defects (L). Recently, exome survey and large-scale re-sequencing confirmed *TRAP1* and *ZIC3* as VATER/VACTERL disease genes. For the majority of affected individuals, the genetic cause remains elusive.

Methods: We performed exome sequencing in a multiplex family previously reported by Hilger et al. (2012). Re-sequencing was performed on an Illumina MiSeq® platform. Candidate gene characterization was performed using embryonic mouse expression studies and zebrafish knockdown experiments.

Results: Exome survey of the index family prioritized a rare variant in the X-chromosome residing gene *SHROOM4* (c.940C>A, p.Glu314Lys). Targeted re-sequencing of 310 male individuals with VATER/VACTERL features and use of GeneMatcher identified two additional families with novel variants in *SHROOM4*. Expression studies in mouse embryos and in zebrafish larvae showed expression in brain, heart, genitourinary tract and developing cloaca. Knockdown experiments in zebrafish larvae using a splice blocking Morpholino revealed cloacal malformations, renal cysts, and heart anomalies. Further analysis showed increased apoptosis in the brain and a higher mortality in comparison with the controls. These phenotypes which strongly resemble the VATER/VACTERL features could be rescued by human wildtype *SHROOM4* RNA.

Conclusions: Previously, variants in *SHROOM4* have been reported in Stocco dos Santos syndrome [MIM #300434] causing intellectual disability. Our genetic and functional data in mouse and zebrafish implicate *SHROOM4* in the expression of a human VATER/VACTERL phenotype.

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P11.132.B A microdeletion on Xq22.1 in a girl with developmental delay and epilepsy may help define a critical region of pathogenicity

Stefano G. Caraffi[1], *Gabriele Trimarchi[1], Sara Giangiobbe[1,2], Chiara Cattani[1], Benedetta Cavirani[1,3], Michela Malacarne[4], Francesca C. Radio[5], Livia Garavelli[1]*

[1]*Azienda USL-IRCCS di Reggio Emilia, Reggio Emilia, Italy,* [2]*San Raffaele Hospital, Milano, Italy,* [3]*Azienda USL di Parma, Parma, Italy,* [4]*IRCCS Istituto Giannina Gaslini, Genova, Italy,* [5]*Ospedale Pediatrico Bambino Gesù, Roma, Italy.*

Deletions in the chromosomal region Xq22 have been associated with intellectual disability, epilepsy and various developmental defects in heterozygous female patients, while they are often lethal in males. Recent reports mainly focus on structural variants encompassing the *PLP1* gene, encoding the major myelin protein, responsible for a variable phenotype in females ranging from late-onset spastic paraplegia to early-onset neurological disease (Hijazi *et al*., Hum Mut 2020;41:150-68). Here we describe a novel female patient presenting with developmental delay, intellectual disability, behavioral anomalies and epilepsy. CGH-array analysis showed a *de novo* 350 Kb deletion in the Xq22.1 region, encompassing the genes *TMSB15A*, *NFX4*, *ARMCX5*, *GPRASP1*, *GPRASP2* and *BHLHB9*. X-chromosome inactivation assay revealed a random inactivation pattern. Various known pathogenic deletions overlap with our patient's, but the majority are larger, sometimes extending into Xq22.2-Xq22.3. The most similar variant

is a 1.1 Mb deletion in a female patient who had clinical features comparable to our case (Grillo *et al*., Eur J Med Genet 2010;53:113-6). The 350 Kb deletion in our patient could therefore represent a critical region within the Xq22.1 locus. Strikingly, a previously published mouse model (Zhou *et al*., Hum Mol Genet 2014;23:3823-9) suggested that the developmental delay and epilepsy phenotype associated with Xq22.1 deletions could be recapitulated in female mice bearing a minimal heterozygous deletion spanning *Armcx5*, *Gprasp1*, *Gprasp2* and *Bhlhb9*. Our report supports the notion of a Xq22.1 microdeletion syndrome not comprising *PLP1* but still associated with severe intellectual disability, and sheds some light on its genotype-phenotype correlations.

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P11.133.C *A ZFHX4* mutation associated with a recognizable neuropsychological and facial phenotype

Paolo Fontana[1], Monia Ginevrino[2,3], Kristel Bejo[2], Giuseppina Cantalupo[1], Maria Ciavarella[1], Cinzia Lombardi[1], Marianna Maioli[1], Francesca Scarano[1], Antonio Novelli[2], ***Fortunato Lonardo[1]***

[1]AORN San Pio, Benevento, Italy, [2]Bambino Gesù Children's Hospital, Roma, Italy, [3]Università Cattolica del Sacro Cuore, Roma, Italy.

Introduction: *ZFHX4* is a gene codifying for a transcription factor involved in the development of various embryonic processes, including brain differentiation. The main features of patients with an 8q21.11 deletion encompassing this gene are intellectual disability, hypotonia, short stature, and a peculiar facial phenotype. Corneal opacity has been frequently reported and some of the subjects also show a wide range of severe eye abnormalities. Case report: We describe a female patient with mild intellectual disability and autism spectrum disorder. She had brachycephaly with flat occiput, wide forehead, strabismus, monolateral ptosis, epicanthic folds, low-set, prominent, posteriorly rotated ears, long and smooth philtrum, thin lips, high-arched palate, microretrognathia, short 4th and 5th metacarpal bones, lumbar hyperlordosis. Clinical Exome Sequencing revealed the presence of the heterozygous *de novo* pathogenic variant c.3093+1G>T in the *ZFHX4* gene. This variant is located in a canonical donor splice site, suggesting a possible alteration of the splicing process and a loss of protein function.

Conclusions: Severe eye abnormalities occur with a high frequency in patients with large 8q21.11 deletions. They range from Peters anomaly to cataract, microphthalmia, sclerocornea, corneal opacity, pigmentary retina degeneration. None of these abnormalities have been reported in patients with *ZFHX4* point mutations, intragenic deletions, or small deletions encompassing only *ZFHX4*. We propose that *ZFHX4* loss-of-function is associated with an autosomal dominant condition, characterized by a neurobehavioural phenotype and a recognizable pattern of facial features. Despite a partial phenotypic overlap, 8q21.11 microdeletion syndrome should be considered as a distinct entity.

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P11.134.D Zhu-Tokita-Takenouchi-Kim syndrome: the first case due to a 21q22.1 microdeletion encompassing *SON* gene

Mafalda Santos Melo*, Susana Lemos Ferreira, Diana Antunes, Rui Gonçalves, Teresa Kay*

Serviço de Genética Médica, Hospital Dona Estefânia, Centro Hospitalar e Universitário de Lisboa Central, Lisboa, Portugal.

Introduction: Zhu-Tokita-Takenouchi-Kim syndrome (ZTTK, OMIM #617140) is a recently described multisystemic disorder caused by *de novo* heterozygous pathogenic variants in *SON* gene. ZTTK syndrome is characterized by developmental delay, poor overall growth, facial dysmorphisms and structural malformations (cleft palate, brain, eye, heart, kidney, and skeletal anomalies). To date, only 35 patients were reported. Here, we present a new case of ZTTK syndrome aiming to contribute to its clinical and mutational spectrum characterization.

Methods: Clinical data was collected from the patient's medical record and compared with literature.

Results: A six-year-old boy was referred for syndromic developmental delay evaluation. He was the first child of a non-consanguineous couple. Previous medical history included congenital heart defect, cleft palate, hypermetropia and recurrent infections. Physical examination showed short stature and facial dysmorphisms including mild frontal bossing, midface retraction, low-set-ears, downslanting palpebral fissures, broad and depressed nasal bridge, full cheeks, short philtrum, small mouth and micrognatia. Abdominal, renal and central nervous system imaging excluded other structural anomalies. Microarray analysis detected a 59.17 kb microdeletion of chromosome region 21q22.1 encompassing *SON* gene (arr[hg19] 21q22.11 (34,892,568-34,951,737)x1).

Discussion: The patient's phenotype was strinkingly similar to ZTTK syndrome. All previous cases were due to loss-of-function mutations in *SON*. To our knowledge, our case was the first associated with a 21q22.1 microdeletion involving a whole *SON* gene deletion. Apparently, the co-deleted genes (*GART, MIR6501* and *DONSON*) did not contribute to the clinical phenotype.

Conclusion: This report confirms that ZTTK syndrome can be caused by 21q22.1 microdeletions, further broadening ZTTK mutational spectrum.

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P12 Cancer Genetics

P12.001.A The copy number loss heterozygosity in hyperdiploid pediatric acute lymphoblastic leukemia

Monika M. Lejman[1]*, Katarzyna Wojciechowska[1], Agata Pastorczak[2], Zuzanna Urbańska[3], Paulina Skowera[4], Borys Styka[4], Anna Pastwińska[5], Joanna Zawitkowska[1], Jerzy R. Kowalczyk[1]*

[1]Medical University of Lublin, Lublin, Poland, [2]Medical University of Lodz, Łódź, Poland, [3]Medical University of Łódź, Łódź, Poland, [4]Children's University Hospital, Lublin, Poland, [5]Medical University of Warsaw, Warszawa, Poland.

Introduction: Genetic abnormalities such as hyperdiploidy and hypodiploidy influence outcome during therapy of childhood B-cell precursor acute lymphoblastic leukemia. Pure trisomies and tetrasomies are the hallmark of hyperdiploid (>47 chromosomes) ALL in children. Studies in ALL show that SNP microarray can reveal a copy-neutral loss of heterozygosity (CN-LOH) of disomic chromosome in hyperdiploid karyotype. Identification of recurrent CN-LOH raises the question of their clinical impact in pediatric leukemia.

Materials and Methods: Between October 2018 and December 2020 400 consecutive children with newly diagnosed BCP-ALL and treated according to AIEOP-BFM ALL 2017 Poland protocol were enrolled into this study. SNP microarray tests (CytoScan HD) and metaphase cytogenetics were performed in patients.

Results: Our analyses identified so far eleven such cases (8 girls, 3 boys). Their chromosome copy number ranged from 49 to 59 and included tetrasomies of chromosomes 21(7x), 18(4x), 10 (2x), X (2x), 14 (1x). We observed CN-LOH of various chromosomes, including chromosomes 1, 3, 11, 13, 15, 16, 20 as a frequently recurrent. All patients continue treatment or remain in remission.

Conclusion: There are no enough comprehensive studies in the literature that reported outcome for patients with CN-LOH of disomic chromosome in hyperdiploid karyotype. Our results indicate that prognostic overview of patients with a CN-LOH hyperdiploid karyotype seems relatively promising. Considering relatively short follow-up, further prospective observation of events in the study cohort is indispensable to assess prognostic value of a CN-LOH hyperdiploid karyotype.

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P12.002.B Clonal architecture of pediatric core binding factor-acute myeloid leukemia (CBF-AML)

Tatiana Nasedkina[1], *Lilit Ghukasyan*[1], *Georgii Krasnov*[1], *Lyudmila Baidun*[2]

[1]*Engelhardt Institute of Molecular Biology of the Russian Academy of Sciences, Moscow, Russian Federation,* [2]*Russian Children's Clinical Hospital, Moscow, Russian Federation.*

Intratumoral heterogeneity and clonal variability is one of the central problems in clinical oncology, being the reason for the development of resistance to therapy and relapses. The use of bioinformatic processing algorithms allows analysis of subclonal tumor organization based on high-throughput sequencing data. In total, 16 patients with t(8;21) and 6 patients with inv(16) were investigated (15 boys and 7 girls, mean age 8.5 years). The paired samples at diagnosis and remission were analyzed, in four patients also relapsed samples were available. Target sequencing of 84 genes, involved in the pathogenesis of AML, or whole-exome sequencing was performed using NextSeq500 Illumina platform. Comparative analysis of target panel and whole-exome sequencing in CBF-AML patients was carried out. Target sequencing showed the presence of several potential driver mutations in *KIT, NRAS, KRAS, CBL, FLT3* signaling pathway genes. In 22% of patients, the presence of two or more signaling mutations with different variant allele frequencies was detected, which may reflect the complex clonal structure of tumor substrate. Additional mutations in *ASXL1, ASXL2, RAD21, ETV6, WT1, SMC3, FBXW7, TET2* were revealed. Analysis of whole-exome sequencing data from the AML patients allowed the isolation of clusters of mutant alleles most likely corresponding to different populations of leukemic cells in the sample. Comparison of the mutation profile of primary AML sample, samples in remission and relapse makes it possible to trace the dynamics of the clonal composition of the tumor. The work was supported by the Russian Science Foundation (grant # 18−15-00398).

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P12.004.D RNA guided CRISPR-Cas protein downregulates the oncogenic driver ALK expression in human lung cancer cell

Saifullah Saifullah[1], *Sakari Matomo*[1], *Takeshi Suzuki*[2,3], *Toshifumi Tsukahara*[1,4]

[1]*Area of Bioscience and Biotechnology, School of Materials Science, Japan Advanced Institute of Science and Technology (JAIST), Nomi City, Ishikawa 923-1292, Japan,* [2]*Division of Functional Genomics, Cancer Research Institute, Kanazawa University, Kakuma-Machi, Kanazawa 920-1192, Japan,* [3]*Molecular Therapeutic Target Research Unit, Institute for Frontier Science Initiative, Kanazawa University, Kakuma-Machi, Kanazawa 920-1192, Japan,* [4]*Division of Transdisciplinary Science, Japan Advanced Institute of Science and Technology (JAIST), Nomi City, Ishikawa 923-1292, Japan.*

Introduction: Anaplastic lymphoma kinase (*ALK*) gene translocation within chromosome 2 results in *EML4-ALK* oncofusion, drivers for lung adenocarcinoma (LUAD). ALK inhibitors like crizotinib have shown tremendous antitumor activity for ALK-positive cancer. However, a complete and long-lasting response to the ALK inhibitor is rare and patients become resistant to the therapy following an initial response. Substitutive therapy that can inhibit overexpression of ALK is desired for precision medicine.

Materials and Methods: Here, we used the CRISPR-Cas13a tool for RNA downregulation. To evaluate the selectivity of CRISPR-Cas13a on guide RNA (gRNA) design, we first knocked down the firefly luciferase mRNA. Next, we simply knocked down the oncogenic driver *EML4-ALK* mRNA in the lung cancer cell (H3122) using the tool. The downregulation was then endorsed by western blot, qPCR, and cell viability assays.

Results: Based on the Cas13a selectivity results, we observed restricted endonuclease activity in 3′ crRNA-gRNA orientation. We found the highest activity between 24-30 bp long gRNAs with limited mismatch tolerance. In the case of EML4-ALK oncofusion, the ALK protein was prominently downregulated (>80%), which was also reflected at the mRNA level. This downregulation resulted in substantial inhibition (40%) of the lung cancer cell viability. We further found that tyrosine phosphorylation was significantly reduced, which is one of the important features for activating the downstream signaling in LUAD. Collectively, this study suggested that the CRISPR-Cas13a protein downregulated the ALK expression in the lung cancer cell.

Conclusion: CRISPR-Cas13a mediated *EML4-ALK* mRNA downregulation could be a potential therapeutic strategy for ALK^{+} lung cancer.

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P12.005.A MDC1 restrains ATR-mediated resection of DNA double-strand breaks in human cells

Stephen Meyn[1,2,3], *Paul S. Bradshaw*[2,3]

[1]*Center for Human Genomics and Precision Medicine, University of Wisconsin, Madison, WI, USA,* [2]*Genetics and Genome Biology, The Hospital for Sick Children, Toronto, ON, Canada,* [3]*Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada.*

Double-strand DNA breaks (DSBs) are a potentially lethal form of DNA damage that can cause genomic instability and contribute to carcinogenesis. Human cells primarily repair induced DSBs via the error-prone classical-Non Homologous End Joining (c-NHEJ) pathway, which occurs throughout the cell cycle. During S and G2, repair also occurs via the error-free Homologous Recombination (HR) pathway. A critical early step in the DNA damage response is resection of DSB ends, which has a major effect on genome stability, as it commits the cell to error-free HR repair.

HR-mediated DSB repair requires the ATM-dependent generation of 3′ single-stranded DNA (ssDNA) via CtIP-mediated DNA end resection. However, we find that DNA end resection can also be regulated by the DNA damage checkpoint protein MDC1 independently of ATM. In human cells, MDC1 loss promotes unrestrained resection of DNA ends, as indicated by increases in the number and intensity of RPA-coated ssDNA foci

in a process that is dependent upon ATR and CtIP. Further, optimal loading of Rad51 onto RPA coated ssDNA requires MDC1. While ATM promotes HR repair by initiating CtIP dependent DNA end resection, MDC1 can affect pathway choice through restraining HR repair by preventing ATR- and CtIP-dependent resection.

Our work identifies a role for MDC1 in the control of DNA end resection in human cells that is independent of ATM and sheds mechanistic insight into the processing of DNA damage that is required for faithful DNA repair, maintenance of genome stability and prevention of tumorigenesis.

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P12.006.B Phenotype of six families with *AXIN2*-oligodontia-colorectal cancer syndrome. Cleft palate as a new feature of this syndrome?

Laura Roht[1,2], *Hanne K. Hyldebrandt*[3], *Astrid T. Stormorken*[3], *Hilde Nordgarden*[4], *Rolf H. Sijmons*[5], *Dennis K. Bos*[5], *Douglas Riegert-Johnson*[6], *Sarah Mantia-Macklin*[6], *Kai Muru*[1,2], *Tiina Kahre*[1,2], *Katrin Õunap*[1,2]

[1]*Department of Clinical Genetics, United Laboratories, Tartu University Hospital, Tartu, Estonia,* [2]*Department of Clinical Genetics, Institute of Clinical Medicine, University of Tartu, Tartu, Estonia,* [3]*Department of Medical Genetics, Oslo University Hospital, Oslo, Norway,* [4]*National Resource Centre for Oral Health in Rare Disorders, Lovisenberg Diaconal Hospital, Oslo, Norway,* [5]*Department of Genetics, University Medical Center Groningen, Groningen, Netherlands,* [6]*Department of Clinical Genomics, Mayo Clinic, Jacksonville, FL, USA.*

Introduction: Oligodontia-colorectal cancer syndrome (OMIM 608615) is an autosomal-dominant disease, which prevalence according to Orphanet is <1: 1,000,000. It is caused by pathogenic germline variants in *AXIN2* gene. Our cohort consists of 13 individuals from six families carrying *AXIN2* disease-causing variants and presenting variable clinical expression of oligodontia-colorectal cancer syndrome.

Aim: To give an overview of our cohort`s clinical phenotype and *AXIN2* gene variants.

Material and methods: Data was gathered through ERN-GENTURIS collaboration and includes five Estonian, five Norwegian, one Dutch and two North-American patients.

Results: Of the 13 *AXIN2* gene variant carriers, 8 were males and 5 were females aged 4-95 years. All were Caucasians, 11 from Europe, two from North-America. The most common symptom was hypodontia/oligodontia and the number of missing teeth ranged from 2-22. Eight of the individuals had some type of gastrointestinal polyps, one individual had adenocarcinoma of the coecum and abdominal superficial melanoma, the other had olfactory neuroblastoma and the third individual had unknown skin cancer and prostate adenocarcinoma. Phenotype was normal in nine cases. In one case, Silver-Russell syndrome was additionally diagnosed and one family had three cases of cleft palate. Frameshift mutations in exon 8 were by far the most frequent and in most cases we know *AXIN2* variant was inherited.

Conclusions: Most of the individual presented some symptoms of oligodontia-colorectal cancer syndrome. In one family, cleft palate was associated with the *AXIN2* pathogenic variant and therefore this characteristic should be considered as part of *AXIN2* phenotype. **Funding:** Estonian Research Council grants PRG471

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P12.008.C *BAP1* germline variations in Finnish patients with malignant mesothelioma

Pauliina Repo[1,2], *Aleksandra Staskiewicz*[1,2], *Eva Sutinen*[3], *Mikko Rönty*[4], *Tero T. Kivelä*[2], *Marjukka Myllärniemi*[3], *Joni A. Turunen*[1,2]

[1]*Folkhälsan Research Center, Helsinki, Finland,* [2]*Ocular Oncology Service, Department of Ophthalmology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland,* [3]*Department of Pulmonary Medicine, Heart and Lung Center, Helsinki University Hospital and Individualized Drug Therapy Research Program, Faculty of Medicine, University of Helsinki, Helsinki, Finland,* [4]*Department of Pathology, HUS Diagnostic Center, Helsinki University Hospital and Faculty of Medicine, University of Helsinki, Helsinki, Finland.*

Pathogenic germline variations in tumour suppressor BRCA1-associated protein 1 (*BAP1*) gene cause a dominantly inherited tumour predisposition syndrome (BAP1-TPDS). Tumours associated with BAP1-TPDS are uveal melanoma (UM), malignant mesothelioma (MM), cutaneous melanoma and renal cell carcinoma. Patients also exhibit cutaneous *BAP1*-inactivated naevi (BIN), the frequency of which is unknown. BINs may arise sporadically, but patients with pathogenic germline *BAP1* variant might harbor multiple BINs even before other tumours. In this study, we sequenced for germline *BAP1* variations 58 DNA samples archived in the Helsinki Biobank from Finnish patients with MM. They were diagnosed in 2010-2019. Sanger sequencing identified one patient (1.7%; 95% CI, 0.04 to 9.2) with a pathogenic variation c.1780_1781insT, p.(G549Vfs*49) in exon 14, a putative founder variant that has been described in five Finnish families with UM. The patient was approximately twenty years younger than the mean of the study cohort when diagnosed with MM (mean 68, range 27 to 82), a nonsmoker with no exposure to asbestos, and without family history of BAP1-TPDS. The tumour showed loss of nuclear BAP1 staining in immunohistochemistry. Additionally, five naevi removed before the MM were analyzed for genetic alterations. Three combined *BAP1*-inactivation with *BRAF* V600E, identified alone in the fourth (compound) naevus, whereas the fifth (intradermal) naevus harbored *NRAS* Q61K. The overall frequency of pathogenic germline *BAP1* variations in Finnish patients with MM was similar to that in Finnish patients with UM (9/433; 2.1%).

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P12.011.C First case of comprehensive genetic diagnostics of Birt-Hogg-Dube syndrome in a Russian patient

Dmitry S. Mikhaylenko[1,2], *Margarita G. Filippova*[3], *Kirill I. Anoshkin*[1], *Nikolay A. Kozlov*[3], *Vsevolod B. Matveev*[3], *Alexander V. Khachaturyan*[3], *Alexandra V. Semyanikhina*[3], *Alexander S. Tanas*[1], *Marina V. Nemtsova*[1,2], *Dmitry V. Zaletayev*[1]

[1]*Research Centre for Medical Genetics, Moscow, Russian Federation,* [2]*I.M. Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russian Federation,* [3]*N.N. Blokhin National Medical Research Center of Oncology, Moscow, Russian Federation.*

Here we report a case study of Birt-Hogg-Dube syndrome (BHDS) in a 26-year-old female patient. The patient was

admitted to a clinic for diagnosis and treatment with a neoplasm of the left kidney and had a history of renal cell cancer (RCC) of the right kidney and spontaneous pneumothorax. Multiple tumors of the left kidney and lung cysts were observed upon clinical and laboratory testing. Tumors of the left kidney were resected and diagnosed by pathologist as chromophobe renal cell carcinomas. PCR and Sanger sequencing of *FLCN* exons 4-14 from blood DNA revealed the heterozygous germline nonsense mutation c.1429C>T (p.R477*) and thus confirmed the diagnosis of BHDS. Multiple fibrofolliculomas, which are the most common BHDS symptoms, were not observed possibly because the patient was too young to develop them. Four variants of uncertain clinical significance were detected in tumor DNA by using the CCP and the IonChef/S5 platform; known cancer driver mutations were not detected. Based on the findings, medical-genetic counselling was carried out, and a follow-up management was outlined. As far as we know, this case study is the first comprehensive clinical and genetic examination of a BHDS patient in Russia. The p.R477* mutation has been described in patients with fibrofolliculomas and lung cysts, but not RCC, while RCC was the first manifestation of BHDS in our case. The case report may help geneticists and oncologists to better understand the clinical and genetic heterogeneity of BHDS in various populations.

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P12.012.D Frequency of pathogenic variants in *BRCA1* and *BRCA2* genes in a Russian population-based sample and in patients with breast or ovarian cancer

Alena Limonova[1], *Alexey Meshkov*[1], *Alexandra Ershova*[1], *Anna Kiseleva*[1], *Olga Skirko*[1], *Marina Klimushina*[1], *Evgeniia Sotnikova*[1], *Olga Kurilova*[1], *Irina Efimova*[1], *Ludmila Lyubchenko*[2], *Margarita Filippova*[2], *Andrey Poloznikov*[3], *Vladimir Kutsenko*[1], *Maria Pokrovskaya*[1], *Oxana Drapkina*[1]

[1]*National Medical Research Centre for Therapy & Preventive Medicine, Moscow, Russian Federation,* [2]*N.N. Blokhin National Medical Research Center of Oncology, Moscow, Russian Federation,* [3]*National Medical Research Radiological Center, Moscow, Russian Federation.*

Introduction: Progress in genetics and molecular research enabled discovery of mutations in BRCA1 and BRCA2, leading to the development of hereditary breast (BC) and ovarian (OC) cancer. Genetic testing contributes to early diagnosis and targeted prevention of these cancers. Objective: To investigate the prevalence of pathogenic variants in BRCA1 and BRCA2 genes in patients with BC/OC and in a population-based sample without oncological diseases.

Materials and Methods: 156 patients (mean age ± SD: 52 ± 13 years old) with diagnosed BC/OC were included in the study consecutively. In addition to it, 672 (mean age ± SD: 45 ± 12 years old) and 1191 (mean age ± SD: 48 ± 11 years old) women from two Russian population-based cohorts of the ESSE-RF study (ESSE-Vologda and ESSE-Ivanovo, respectively) were recruited in the analysis. Variants of BRCA1 (rs80357713, rs80357711, and rs80357906) and BRCA2 (rs80359550) were detected using a custom panel.

Results: Among cancer patients there were 5 carriers (3.21%) of mutations in BRCA1 (4 - rs80357906, 2.56%; 1 - rs80357711, 0.64%) and 1 carrier (0.64%) of rs80359550 in BRCA2. In the population-based samples no mutations were identified. The presence of BRCA1 (rs80357906, rs80357711) or BRCA2 (rs80359550) variants increases the risk of BC/OC statistically significantly (<0,0001). As no carriers were detected in the population-based samples, only lower limit of confidence interval for OR was calculated and was found to be 14,4.

Conclusion: Detection of pathogenic variants in BRCA1 and BRCA2 could facilitate early diagnosis and timely prevention of BC and OC.

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P12.013.A A 15 and 28 Gene Panel for BRCA1, BRCA2 and DDR Genes for Reporting Variants on FFPE Samples

Fiona Hyland[1], *Charles Scafe*[1], *Yun Zhu*[2], *Chenchen Yang*[2], *Yu-Ting Tseng*[2], *Steven Roman*[2]

[1]*Thermo Fisher, South San Francisco, CA, USA,* [2]*Thermo Fisher, San Mateo, CA, USA.*

There is broad interest in detection of germline and somatic mutations in BRCA1 and BRCA2 and other DNA damage response (HR DDR) genes, including detect SNPs, Indels, CNVs, and exon deletions on FFPE samples.We describe analytical verification of 15-gene and 28-gene DDR next-generation sequencing assays, covering *BRCA1* and *BRCA2* and additional DDR genes. These panels can be customized, adding up to 250 optimized and performance verified genes. These panel detects germline and somatic mutations on FFPE samples, with sensitive and specific detection of variants down to 5% LOD. Exon deletions and long deletions are reported for *BRCA1* and *BRCA2*. The panels perform well with low input (20ng) and degraded DNA.The Oncomine BRCA Expanded Panel has excellent performance. 92% of reads are on target, and panel uniformity is 97%. Panel uniformity at hotspot positions (alleles of known relevance) ranges from 98-100%. Performance was measured with the Ion GeneStudio S5 system, with FFPE samples and cell line controls. An integrated bioinformatics pipeline with a visual user interface provides variant calling, functional annotation of variants, presence in population, phenotype and oncology databases including ClinVar, COSMIC etc, and predicted protein effect. Filtering tools enable variant prioritization. We create a report describing drug labels and clinical trials relevant for the variants detected in the sample.

This assay enables *BRCA1/2* and HR DDR translational research into the effects of relevant mutations. For Research Use Only. Not for use in diagnostic procedures

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P12.014.B Harnessing national pan-laboratory data for accurate quantitation of PS4 in cancer susceptibility genes: Cancer Variant Interpretation Group UK (CanVIG-UK)

Alice Garrett[1], *Chey Loveday*[1], *Miranda Durkie*[2], *George J. Burghel*[3], *James Drummond*[4], *Rachel Robinson*[5], *Ian Berry*[5], *Andrew Wallace*[3], *Laura King*[1], *Subin Choi*[1], *Eleni Sofianopoulou*[6], *Fiona McRonald*[7], *Francesco Santaniello*[7], *John Burn*[8], *Jem Rashbass*[9], *Steven Hardy*[7],

Diana Eccles[10,11], Mark Tischkowitz[12,13], Clare Turnbull[1,14], on behalf of CanGene-CanVar consortium and CanVIG-UK

[1]Division of Genetics and Epidemiology, Institute of Cancer Research, London, United Kingdom, [2]Yorkshire and North East Genomic Laboratory Hub, Sheffield Children's NHS Foundation Trust, Sheffield, United Kingdom, [3]Manchester Centre for Genomic Medicine and North West Genomic Laboratory Hub, Manchester University NHS Foundation Trust, Manchester, United Kingdom, [4]East Genomic Laboratory Hub, Cambridge University Hospitals Genomic Laboratory, Cambridge University Hospitals, Cambridge, United Kingdom, [5]Yorkshire and North East Genomic Laboratory Hub, Leeds Teaching Hospitals NHS Trust, Leeds, United Kingdom, [6]Department of Public Health and Primary Care, Clinical Medicine, University of Cambridge, Cambridge, United Kingdom, [7]National Disease Registration, Public Health England, London, United Kingdom, [8]Institute of Genetic Medicine, International Centre for Life, Newcastle upon Tyne, United Kingdom, [9]National Cancer Registration and Analysis Service, Public Health England, London, United Kingdom, [10]Cancer Sciences, Faculty of Medicine, University of Southampton, Southampton, United Kingdom, [11]Human Genetics and Genomic Medicine, Faculty of Medicine, University of Southampton, Southampton, United Kingdom, [12]East Anglian Medical Genetics Unit, Cambridge University Hospitals NHS Trust, Cambridge, United Kingdom, [13]Department of Medical Genetics, National Institute for Health, Research Cambridge Biomedical Research Centre, University of Cambridge, Cambridge, United Kingdom, [14]Cancer Genetics Unit, Royal Marsden NHS Foundation Trust, London, United Kingdom.

For genes associated with incomplete, late-onset penetrance for common phenotypes, the lines of evidence available for inference of pathogenicity are often limited. Individual labs in isolation typically lack sufficient data to undertake informative case-control analyses. Given the national infrastructure of the NHS and strong professional cohesion afforded by ACGS/BSGM, the UK is well-placed to address this issue through amalgamation of data, but has been perennially limited by issues of governance and infrastructure.

Precipitated in 2016 by the BRCA Challenge, all 19 English NHS molecular genetics laboratories now regularly submit pseudonymised individual-level variant data to the National Cancer Registration and Analysis Service of Public Health England (PHE). Via the CRUK-supported CanGene-CanVar initiative, these data are harmonised, cleaned and ethnicity-matched in PHE. In total to date we have had submissions for >100,000 BRCA tests and >20,000 CRC/MMR gene tests. Variant counts are then released back to the clinical community via an online portal (http://www.canvaruk.org/).

We shall present UK NHS data on analysis of >1500 BRCA variants to exemplify our proposed approach to graded quantitative application of ACMG evidence item PS4, namely use of variant-level case control data. We shall demonstrate key elements to our PS4 approach, including (i) how to approximate an appropriate control denominator when a variant is absent in gnomAD (ii) how to derive from an odds ratio of association a likelihood ratio for pathogenicity and (iii) adjustment for sample series enrichment incurred from recruitment based on family history.

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P12.015.C Clinical practice guidelines for *BRCA1* and *BRCA2* genetic testing

Marion Imbert-Bouteille[1], *Massimo Barberis[2], Philip Beer[3], Eitan Friedman[4], Josep M. Piulats[5], Ettore D. Capoluongo[6], Jesus Garcia Foncillas[7], Isabelle Ray-Coquard[8], Frédérique Penault-Llorca[9], William D. Foulkes[10], Clare Turnbull[11], Helen Hanson[11], Steven Narod[12], Banu K. Arun[13], Matti S. Aapro[14], Jean-Louis Mandel[15], Nicola Normanno[16], Diether Lambrechts[17], Ignace Vergote[18], Bernard Baertschi[19], Karen Baudry[1], Yves-Jean Bignon[9], Marc Bollet[20], Carole Corsini[1], Olivier Cussenot[21], Thibault De la Motte Rouge[22], Marie Duboys de Labarre[1], Florence Duchamp[1], Clarisse Duriez[23], Karim Fizazi[24], Virginie Galibert[1], Laurence Gladieff[25], Joseph Gligorov[21], Pascal Hammel[26], William Jacot[27], Tatiana Kogut-Kubiak[1], Pierre-Jean Lamy[28], Sophie Nambot[29], Yann Neuzillet[30], Sylviane Olschwang[31], Jean-Marc Rey[1], Chloé Rideau[1], Jean-Philippe Spano[32], Frédéric Thomas[33], Marion Vandromme[23], Julie Vendrell[1], Daniel Zarca[34], Kevin S. Hughes[35], José E. Alés Martínez[36], Pascal Pujol[1]*

[1]CHU Montpellier, MONTPELLIER, France, [2]European Institute of Oncology, MILAN, Italy, [3]University of Glasgow, GLASGOW, United Kingdom, [4]Chaim Sheba Medical Center, TEL-HASHOMER, Israel, [5]Institut Català d'Oncologia (ICO), HOSPITALET DE LLOBREGAT, Spain, [6]Federico II School of Medicine, NAPLES, Italy, [7]Oncohealth Institute, MADRID, Spain, [8]Centre Léon Bérard, LYON, France, [9]Centre Jean Perrin, CLERMONT-FERRAND, France, [10]The Research Institute of the McGill University, MONTREAL, QC, Canada, [11]The Institute of Cancer Research, LONDON, United Kingdom, [12]Women's College Research Institute, TORONTO, ON, Canada, [13]University of Texas MD Anderson Cancer Center, HOUSTON, TX, USA, [14]Clinique de Genolier, GENOLIER, Switzerland, [15]The Institute of Genetics and Molecular and Cellular Biology, STRASBOURG, France, [16]Istituto Nazionale Tumori "Fondazione G. Pascale", NAPLES, Italy, [17]VIB Center for Cancer Biology, LEUVEN, Belgium, [18]Catholic University of Leuven, LEUVEN, Belgium, [19]University of Geneve, GENEVE, Switzerland, [20]Institut Rafaël, LEVALLOIS-PERRET, France, [21]Hôpital Tenon AP-HP, PARIS, France, [22]CRLCC Eugène Marquis, RENNES, France, [23]Association BRCA France, LILLE, France, [24]Institut Gustave Roussy, PARIS, France, [25]Institut Claudius Regaud, TOULOUSE, France, [26]Hôpital Beaujon AP-HP, MONTPELLIER, France, [27]Institut du Cancer de Montpellier, MONTPELLIER, France, [28]Institut Imagenome, MONTPELLIER, France, [29]CHU Dijon, DIJON, France, [30]Hôpital Foch, SURESNES, France, [31]Hôpital Européen Marseille, MARSEILLE, France, [32]Hôpital Pitié-Salpêtrière AP-HP, PARIS, France, [33]CNRS Montpellier, MONTPELLIER, France, [34]Institut Français du Sein, PARIS, France, [35]Massachusetts General Hospital, BOSTON, MA, USA, [36]Hospital Nuestra Senora de Sonsoles, AVILA, Spain.

BRCA1 and BRCA2 gene pathogenic variants account for most hereditary breast cancer and are increasingly used to determine eligibility for PARP inhibitor (PARPi) therapy of BRCA-related cancer. Because issues of BRCA testing in clinical practice now overlap with both preventive and therapeutic management, updated and comprehensive practice guidelines for BRCA genotyping are needed. The integrative recommendations for BRCA testing presented here aim to 1) identify individuals who may benefit from genetic counseling and risk-reducing strategies; 2) update germline and tumor-testing indications for PARPi-approved therapies; 3) provide testing recommendations for personalized management of early and metastatic breast cancer; and 4) address the issues of rapid process and tumor analysis. An international group of experts including geneticists, medical and surgical oncologists, pathologists, ethicists and patient representatives was commissioned by the French Society of Predictive and Personalized Medicine. The group followed a methodology based on specific formal guidelines development including 1) evaluating the likelihood of BRCAm from

a combined systematic review of the literature, risk assessment models and expert quotations and 2) therapeutic values of BRCAm status for PARPi therapy in BRCA-related cancer and for management of early and advanced breast cancer. These international guidelines may help clinicians comprehensively update and standardize BRCA testing practices.

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P12.016.D Uptake and efficacy of bilateral risk reducing surgery in unaffected female BRCA1 and BRCA2 carriers

Ruta Marcinkute[1], *Emma Roisin Woodward*[1], *Ashu Gandhi*[2], *Sacha Howell*[2], *Emma J Crosbie*[3], *Julie Wissely*[2], *James Harvey*[2], *Lindsay Highton*[2], *John Murphy*[2], *Cathrine Holland*[4], *Richard Edmondson*[4], *Richard Clayton*[4], *Lester Barr*[2], *Elaine F Harkness*[5], *Anthony Howell*[6], *Fiona Lalloo*[1], *D Gareth Evans*[1]

[1]*Clinical Genetics Service, Manchester Centre for Genomic Medicine, Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom,* [2]*Prevent Breast Cancer Centre, Wythenshawe Hospital Manchester Universities Foundation Trust, Manchester, United Kingdom,* [3]*Division of Cancer Sciences, Faculty of Biology, Medicine and Health, St Mary's Hospital, University of Manchester, Manchester, United Kingdom,* [4]*Department of Obstetrics and Gynaecology, St Mary's Hospital, Manchester University NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester, United Kingdom,* [5]*Division of Informatics, Imaging and Data Sciences, School of Health Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester Academic Health Science Centre, Manchester, United Kingdom,* [6]*Manchester Breast Centre, The Christie Hospital, Manchester, United Kingdom.*

Background: Women testing positive for *BRCA1/2* pathogenic variants have high lifetime risks of breast cancer (BC) and ovarian cancer (OC). The effectiveness of risk reducing surgery (RRS) has been demonstrated in numerous previous studies. We evaluated long-term uptake, timing and effectiveness of risk reducing mastectomy (RRM) and bilateral salpingo-oophorectomy (RRSO) in healthy *BRCA1/2* carriers.

Methods: Women were prospectively followed up from positive genetic test (GT) result to censor date. χ^2 testing compared categorical variables; Cox regression model estimated HRs and 95% CI for BC/OC cases associated with RRS, and impact on all-cause mortality; Kaplan-Meier curves estimated cumulative RRS uptake. The annual cancer incidence was estimated by women-years at risk.

Results: In total, 887 women were included in this analysis. Mean follow-up was 6.26 years (range = 0.01-24.3; total = 4685.4 women-years). RRS was performed in 512 women, 73 before GT. Overall RRM uptake was 57.9% and RRSO uptake was 78.6%. The median time from GT to RRM was 18.4 months, and from GT to RRSO-10.0 months. Annual BC incidence in the study population was 1.28%. Relative BC risk reduction (RRM versus non-RRM) was 94%. Risk reduction of OC (RRSO versus non-RRSO) was 100%.

Conclusion: Over a 24-year period, we observed an increasing number of women opting for RRS. We showed that the timing of RRS remains suboptimal, especially in women undergoing RRSO. Both RRM and RRSO showed a significant effect on relevant cancer risk reduction. However, there was no statistically significant RRSO protective effect on BC.

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P12.020.D Nearly all multiple-case breast cancer families with HRD tumors are detected by clinical gene panels

Thibaut S. Matis[1,2,3], *Nadia Zayed*[2,3], *Bouchra Labraki*[4], *Manon de la Durantaye*[5], *Théophane A. Matis*[6], *Nancy Hamel*[3], *Adrienne Atayan*[2], *Barbara Rivera*[7,8,9], *Yuval Tabach*[10], *Patricia N. Tonin*[2,3,11], *Alexandre Orthwein*[7,8], *Anne-Marie Mes-Masson*[5,12], *Zaki El Haffaf*[4], *William D. Foulkes*[2,3,7], *Paz Polak*[13]

[1]*Institut Bergonié, Bordeaux, France,* [2]*Department of Human Genetics, McGill University, Montréal, QC, Canada,* [3]*Cancer Research Program, Centre for Translational Biology, The Research Institute of the McGill University Health Centre, Montréal, QC, Canada,* [4]*Divison de Médecine Génétique, CRCHUM-CHUM, Montréal, QC, Canada,* [5]*Centre de recherche du Centre hospitalier de l'Université de Montréal and Institut du cancer de Montréal, Montréal, QC, Canada,* [6]*Ecole Supérieur de Génie Informatique, Paris, France,* [7]*Gerald Bronfman Department of Oncology, McGill University, Montréal, QC, Canada,* [8]*Lady Davis Institute for Medical Research, Jewish General Hospital, Montréal, QC, Canada,* [9]*IDIBELL Bellvitge Biomedical Research Institute, Barcelona, Spain,* [10]*Department of Developmental Biology and Cancer Research, Institute for Medical Research-Israel-Canada, Hebrew University of Jerusalem, Jerusalem, Israel,* [11]*Department of Medicine, McGill University, Montréal, QC, Canada,* [12]*Department of Medicine, Université de Montréal, Montréal, QC, Canada,* [13]*Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai Hospital, New York, NY, USA.*

Germline pathogenic variants (GPVs) in *BRCA1*, *BRCA2*, *PALB2*, and *RAD51C/D* account for about two-fifths of multi-case breast cancer (BC) families. Because the known germline pathogenic variants are mostly related to homologous recombination repair (HR) pathway genes, it has been hypothesized that variants in unknown genes or variants of uncertain significance that lead to HR deficiency (HRD) can contribute to a large fraction of the unsolved cases. Through a pipeline optimized for archival formalin-fixed paraffin-embedded tumor tissues, we studied 39 whole-exome sequencings normal/tumor pairs from French-Canadian patients with either early-onset BC or a strong family history of BC negative for known breast cancer susceptibility genes (BCSGs). Consistent with previous studies, less than 15% of tumors demonstrated HRD, and one of which harbored a founder missense mutation in *RAD51D*, recently classified as pathogenic. No other samples revealed novel candidate HRD-associated genes, selected on their co-evolution with HR genes. Thirteen patients harbored loss of heterozygosity in the matched tumor that retained variants in a candidate gene, but tumors did not have a mutational signature indicating HRD. We showed that our pipeline could be used to identify rare GPV that lead to HRD, but our results suggest that in French Canadians it is unlikely that a significant fraction of unexplained multi-case families is due to HRD-associated variants. This pipeline will be useful for investigating understudied populations. In well-studied populations, these results suggest that current gene testing panels will be able to detect most pathogenic variants in the known cancer-predisposing HRD genes.

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P12.021.A The clinical significance of combined effect in *BRCA1* and *p53* genes variants on breast cancer treatment course

Olha Lobanova[1], *Z Rossokha*[2], *V Cheshuk*[1], *N Medvedieva*[2], *R Vereshchako*[1], *N Gorovenko*[3]

[1]*National Medical University Bogomolets, Kyiv, Ukraine,* [2]*State Institution Reference-centre for Molecular Diagnostic of Public Health Ministry of Ukraine, Kyiv, Ukraine,* [3]*Shupyk National Medical Academy of Postgraduate Education, Kyiv, Ukraine.*

Introduction: *BRCA 1/2* genes identified as hereditary determinants of a high risk breast cancer (BC). The pathogenic variants in *BRCA* genes is detected in familial BC, in contrast the benign and drug response variant with uncertain significance in *p53* gene (G119C). The aim of our study was to evaluate the combine effect of these gene variants on the clinical course of the disease.

Materials and Methods: The study group included 165 BC patients with a follow-up period of 28-36 months. Variants in the *BRCA1* gene (*5382insC*, rs80357906; 4153delA, rs80357711; T300G, rs28897672) and in the *p53* gene *(*G119C, rs1042522) were detected by PCR and PCR-RFLP.

Results: *BRCA1* pathogenic variants were found in 12.7% cases, *p53* single variants - in 52.1 %. 5,5 % patients had combined variants in pathogenic *BRCA1* with variants of *P53* gene (5382insC/G119C - 4,8%; 4153delA/G119C - 0,6%). The *p53* single variants was defined in 76.7% of familial BC. Triple-negative BC type detected in 37% of all patients. Among triple-negative patients with pathogenic *BRCA1* was 81%, with the *p53* variants - 36%, with combined variants of two genes - 100% patients. Disease-free survival and overall survival was decreased in patients with combined variants compared with other patients (44.4% vs 87.3%; 66.7 % vs 96.4%, $p < 0.05$, respectively). Brain metastases were identified more often in patients with combined genes variant (60% vs 28,6%, $p < 0.05$, respectively).

Conclusions: The unfavorable effect of combined *BRCA1* and *p53* gene variants with aggressive course and resistance to treatment in patients with breast cancer was defined.

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P12.022.B Case-control likelihood ratio calculation for clinical classification of variants of uncertain significance in the *BRCA1* and *BRCA2* genes

Maria Zanti[1], *Fergus Couch*[2], *Denise O'Mahony*[1,3], *Breast Cancer Association Consortium, Joe Dennis*[4], *Douglas Easton*[4], *Amanda Spurdle*[5], *David Goldgar*[6], *Kyriaki Michailidou*[1,3]

[1]*Biostatistics Unit, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus,* [2]*Mayo Clinic, Rochester, MN, USA,* [3]*Cyprus School of Molecular Medicine, Nicosia, Cyprus,* [4]*Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom,* [5]*Division of Genetics and Population Health, QIMR Berghofer Medical Research Institute, Brisbane, Australia,* [6]*Huntsman Cancer Institute, University of Utah School of Medicine, Department of Dermatology, Salt Lake City, UT, USA.*

Introduction: It is well established that the most prevalent inherited pathogenic variants (PVs) contributing to breast cancer (BC) risk occur in the *BRCA1* and *BRCA2* genes. Individuals carrying PVs can benefit from risk management strategies including closer surveillance at an earlier age, prophylactic surgery and targeted therapies. However, *BRCA1* and *BRCA2* genetic testing often (5-20%) identifies a variant of uncertain clinical significance (VUS), complicating clinical management of patients and their families.

Materials and Methods: After quality control, genotype data generated as part of the Breast Cancer Association Consortium (BCAC) OncoArray project was available for 184 *BRCA1/2* variants. Data from up to 75,657 BC cases and 52,987 healthy controls (ages between 18-80) were used to estimate likelihood ratios (LRs) of pathogenicity. LRs were calculated by comparing the probability of observing the data assuming the variant has similar penetrance as the "average" truncating pathogenic variant with the probability that the variant under study has no effect on risk. LRs were categorised into American College of Medical Genetics and Genomics and Association for Molecular Pathology (ACMG/AMP) code strength categories following published recommendations.

Results: Our analysis provides supporting, moderate or strong classification evidence for 70.1% of the variants. Of these, LR estimates in favour of pathogenicity were estimated for 17 variants, whereas LRs providing evidence of being benign were estimated for 112 variants.

Conclusions: These results may be used to inform *BRCA1* and *BRCA2* variant classification, with potential implications for patient management. Grant: Research Promotion Foundation; RESTART 2016 - 2020; CULTURE/AWARD-YR/0418/0017

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P12.023.C Association of XRCC1-Arg399Gln and XPG-Asp1104His polymorphisms with reproductive risk factors and increased risk of breast cancer in Tanzanian patients

Ismael C. Adolf[1], *Gokce Akan*[2], *Linus P. Rweyemamu*[1], *Fatmahan Atalar*[2]

[1]*University of Dar es Salaam Mbeya College of Health and Allied Sciences, Mbeya, Tanzania, United Republic of,* [2]*Child Health Institute, Department of Rare Diseases, Istanbul University, Istanbul, Turkey, Istanbul, Turkey.*

Background: Variations in DNA repair genes can alter protein function, resulting in the accumulation of DNA damage and mutations, and contribute to the development of cancer. Recent findings have shown the association between DNA repair gene polymorphisms and pathogenesis of breast cancer(BC). In this study, we investigated the role of the following polymorphisms; hOGG1-Ser326Cys(rs1052133), APE1-Asp148Glu(rs1130409), XRCC1-Arg399Gln(rs25487), XPG-Asp1104His(rs17655), XPD-Lys751Gln(rs13181), hMSH2-Gly322Asp(rs4987188), XRCC3-Thr241Met(rs861539), XRCC2-Arg188His(rs3218536), RAD51-4719A/T(rs2619679) and RAD51-4601A/G(rs5030789) in susceptibility in BC in Tanzania. The impact of reproductive risk factors on BC was also evaluated.

Methods & Results: A hospital based case-control study was carried out in Tanzanian population(263 BC patients and 250 controls matched for sex and age). QRT-PCR was used for genotyping assay. Allelic, genotypic and haplotype association analyses with disease risk and reproductive risk factor were performed. The frequency of genotypic and allelic variants of XRCC1-Arg399Gln, XRCC2-Arg188His, XRCC3-Thr241Met, XPG-Asp1104His and MSH2-Gly322Asp were significantly different between the groups($p < 0.05$,respectively). Moreover, XRCC1-Arg399Gln, XRCC3-Thr241Met and XPG-Asp1104His polymorphisms were associated with the increased risk of BC in dominant, recessive and co-dominant genetic-inheritance models($p < 0.05$, respectively). Association analysis between reproductive risk factors and DNA repair gene polymorphisms revealed that XRCC1-Arg/Gln genotypes had 3.1-fold increased risk of BC in pre-menopausal patients compared to their post-menopausal counterpart($p = 0.001$). Also, XPG-His/His genotypes had 1.2-fold increased risk of BC in younger BC patients(<40years)compared to older patients(≥40years)($p = 0.028$). Moreover, XPG-Asp1104His was associated with Luminal-A subtype and PR-positivity($p = 0.042$, $p = 0.021$, respectively). And, MSH2-Gly322Asp was significantly associated with HER2-positivity($p = 0.028$) in Tanzanian BC patients.

Conclusion: Our studies may well confirm that XRCC1-Arg399Gln and XPG-Asp1104His polymorphisms may influence the reproductive risk factors and increased risk of BC in Tanzania.

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P12.024.D Pathogenic Variants in Hereditary Cancer Syndrome Genes are Prevalent Among Breast Cancer Patients Not Meeting Various International Genetic Testing Guidelines

Sarah M. Nielsen[1], *Emily Decker*[1], *Natalie Rickers*[1], ***Alekhya Narravula***[1], *Pat W. Whitworth*[2], *Peter D. Beitsch*[3], *Edward D. Esplin*[4], *Robert L. Nussbaum*[1]

[1]*Invitae Corp., San Francisco, CA, USA,* [2]*Nashville Breast Center, Nashville, TN, USA,* [3]*Dallas Surgical Group, Dallas, TX, USA,* [4]*Invitae Corp., Chicago, IL, USA.*

Background: Clinical management options have expanded for patients harboring pathogenic variants (PVs) in cancer predisposition genes. Historically, testing costs and clinical implementation challenges led to restrictive testing guidelines in many countries. Increasing evidence demonstrates that broader testing is a cost-effective way to identify patients with PVs. We assessed the efficacy of multiple international testing guidelines in identifying breast cancer (BC) patients with clinically actionable PVs.

Methods: We reanalyzed a prospective cohort of U.S.-based, primarily Northern European, BC patients, referred for germline genetic testing (PMID: 30526229). We applied testing guidelines from Australia, U.K. and 2 Canadian provinces (Ontario, British Columbia) to determine their sensitivity for selecting patients with PVs in high risk (>4x risk compared to general population) breast/ovarian cancer genes. These populations were chosen because of similar healthcare systems and ancestral distribution.

Results: Table 1 displays the distribution of in criteria (IC) vs. out of criteria (OOC) patients by testing criteria. Over 75% of patients analyzed were OOC. Rates of PVs were similar between IC and OOC patients. Existing criteria missed up to 30% of patients with high risk PVs. The majority (>80%) of PVs in OOC patients were in genes with published management guidelines.

Conclusions: In our cohort, select international testing criteria identified <30% of patients with PVs. These data suggest expanding certain international guidelines would allow better identification and improved management for BC patients across the globe.

Findings in IC vs. OOC patients US, United States; B.C., British Columbia; UK, United Kingdom NCCN,

		Overall					In criteria	Out of criteria
Country/ providence	Guideline	Total n of cohort	IC (% of total cohort)	OOC (% of total cohort)	Total PV IC (% of IC cohort)	Total PV OOC (% of OOC cohort)	High risk^ PVs (% of total PVs)	High risk^ PVs (% of total PVs)
U.S.	NCCN	953	473 (49.6)	480 (50.4)	43 (9.1)	40 (8.3)	22 (26.5)	8 (9.6)
Ontario	MOHLTC	953	210 (22.0)	743 (78.0)	18 (8.6)	65 (8.7)	5 (6.0)	25 (30.1)
B.C.	BCHCP	953	203 (21.3)	750 (78.7)	24 (11.8)	59 (7.9)	9 (10.8)	19 (22.9)
Australia	eviQ	953	180 (18.9)	773 (81.1)	19 (10.6)	64 (8.3)	12 (14.5)	18 (21.7)
U.K.	NICE*	826**	127 (14.7)	736 (85.3)	11 (8.7)	64 (8.7)	6 (7.2)	22 (26.5)

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P12.025.A Impact of functional polymorphisms in oxidative stress-related genes on early-stage breast cancer prognosis

Erika Korobeinikova, *Rasa Ugenskiene, Ruta Insodaite, Elona Juozaityte*

Lithuanian University of Health Sciences, Kaunas, Lithuania.

Introduction: Oxidative stress-related proteins NFE2L2, HMOX1 and TXNRD2 play a role in breast cancer (BC) pathogenesis. Functional single nucleotide polymorphisms (SNPs) in their coding genes may alter protein levels and impact the course of BC. We aimed to evaluate possible associations of *NFE2L2* rs10183914, rs35652124, *HMOX1* rs2071746, *TXNRD2* rs1139793 SNPs with the early-stage BC clinicopathological characteristics and survival.

Materials and methods: study involved 202 Eastern European (Lithuanian) women with primary I-II stage BC. DNA was extracted from peripheral blood leukocytes. Alleles were genotyped using TaqMan genotyping assays. Association of clinicopathological characteristics (age at diagnosis: ≤50/>50 years; tumour size: ≤2cm/>2-5 cm; lymph node, oestrogen receptor, progesterone receptor and HER2 status: positive/ negative; tumour differentiation grade: G1+G2/G3) and SNPs was evaluated using $\chi 2$ test. Univariate differences between disease-free survival (DFS), metastasis-free survival (MFS) and overall survival (OS) rates were tested for significance using the log-rank test; while multivariate analysis for survival was tested using Cox proportional hazards models. Multivariate analysis included above mentioned pathomorphological characteristics. $p < 0.05$ was considered statistically significant.

Results: No associations between clinicopathological variables and SNPs were observed. In both, univariate and multivariate Cox survival analysis, *TXNRD2* rs1139793 GG genotype vs. GA+AA was a negative prognostic factor for DFS (multivariate HR 2.248, $p = 0.025$) and OS (multivariate HR 2.248, $p = 0.029$).

Conclusions: *TXNRD2* rs1139793 polymorphism may contribute to the identification of early-stage BC patients at a higher risk for disease recurrence and death. Further investigations with larger sample sizes are needed to confirm the results of the current study.

E. Korobeinikova: None. **R. Ugenskiene:** None. **R. Insodaite:** None. **E. Juozaityte:** None.

P12.026.C Clinical utility of the 313-variant based polygenic risk score and multigene panel testing for breast cancer risk prediction

Inge M. M. Lakeman[1], *Mar Rodriguez-Girondo*[1], *Andrew Lee*[2], *Antoinette Hollestelle*[3], *Marjanka K. Schmidt*[4,1], *Christi J. van Asperen*[1], *Peter Devilee*[1], *on behalf of the HEBON study*

[1]*Leiden University Medical Centre, Leiden, Netherlands,* [2]*University of Cambridge, Cambridge, United Kingdom,* [3]*Erasmus MC Cancer Institute, Rotterdam, Netherlands,* [4]*Netherlands Cancer Institute, Amsterdam, Netherlands.*

Introduction: We explored the BOADICEA model's clinical utility, which currently incorporates the effects of truncating variants in 5 breast cancer genes, family history, and a polygenic risk score (PRS) based on 313 common variants, in non-*BRCA1/2* Dutch breast cancer families.

Materials and Methods: We applied logistic regression to estimate the association of PRS-313 with breast cancer risk using 3,925 breast cancer cases from 3,528 non-*BRCA1/2* breast cancer families and 3,479 population controls. Lifetime risks of cases were retrospectively calculated using BOADICEA, simulating an individual to be at the age of one year and unaffected.

Results: We found a significant association between PRS-313 and breast cancer with and without adjustment for family history based on pedigrees, per SD OR = 1.54, 95%CI[1.31-1.80] and OR = 1.96, 95%CI[1.84-2.09] respectively. Similar results were found for known ER-positive and ER-negative tumours separately. Applying risk classification thresholds from guidelines in the USA, United Kingdom and the Netherlands (NCCN, NICE, and IKNL), risk assessment by including the PRS-313 to BOADICEA would have changed screening recommendations in *CHEK2* and *ATM* pathogenic variant carriers to a similar degree as non-carriers, but not in *PALB2* pathogenic variant carriers (Table).

Conclusions: Our results demonstrate that use of the PRS-313 in the BOADICEA model in genetically unexplained breast cancer families and carriers of moderate breast cancer risk variants can potentially influence clinical management in substantial proportions of counselees.

		Number	Percentage of women that would change breast cancer risk classification after addition of PRS-313 to BOADICEA IKNL guideline		
			IKNL guideline	NICE guideline	NCCN guideline
All cases		3,925	34	36	27
All controls		3,479	5	11	5
Cases with gene panel result	Non-carriers	2,313	34	34	27

	Number	Percentage of women that would change breast cancer risk classification after addition of PRS-313 to BOADICEA IKNL guideline		
		IKNL guideline	NICE guideline	NCCN guideline
ATM carriers	40	23	23	0
CHEK2 carriers	164	27	24	7
PALB2 carriers	10	0	0	0

I.M.M. Lakeman: None. **M. Rodriguez-Girondo:** None. **A. Lee:** None. **A. Hollestelle:** None. **M.K. Schmidt:** None. **C.J. van Asperen:** None. **P. Devilee:** None.

P12.027.C Mendelian randomization and colocalization study revealing splicing-type specific effects on cancers

Huiling Zhao, *Philip.C. Haycock, Tom R. Gaunt, Jie Zheng*

MRC Intergrative Epidemiology Unit, Bristol University, Bristol, United Kingdom.

Changes in alternative splicing patterns can lead to partial or total loss of protein functional domains, thereby affecting tumorigenesis, progress and prognosis. However, the contribution of alternative splicing to cancer development is unclear.

We applied two-sample Mendelian randomization (MR) and colocalization to detect splicing events with putative causal roles on breast, lung, ovarian and prostate cancer, with 9,230 sQTLs representing 9,585 splicing events across 49 tissues from healthy GTEX samples as genetic instruments. The splicing-type specific effects of our top MR findings were further validated using sQTLs from CancersplicingQTL database, with samples from cancer patients.

Among 618,932 splicing event-cancer MR analyses using GTEX data, 2,412 associations showed robust MR and colocalization evidence (MR_P < 5e-8, colocalization probability>0.8), which were set as reliable findings. In a tissue specific manner, 746 MR associations (31%) were observed in the same tissue types of the corresponding cancers. For example, a splicing event of *MAN2C1* has a causal effect on breast cancer risk only in breast tissue. We validated the top splicing event-cancer associations across six splicing types in CancersplicingQTL and found that the top MR associations showed different splicing-type enrichment patterns across different cancers. Alternative donor sites accounted for 6% of all potential effects on lung cancer, and 82% for top lung cancer MR findings. Similarly, Exon Skip showed splicing-type enrichment on top prostate cancer MR associations.

Our study suggests that different splicing types may have different enrichment patterns of causal effects on different cancers. This provides information for mechanism exploration and drug target prioritization.

H. Zhao: None. **P. Haycock:** None. **T. Gaunt:** None. **J. Zheng:** None.

P12.029.A Association of *VEGF* Haplotypes with Breast cancer risk in North-West Indians

Kamlesh Guleria[1], *Vasudha Sambyal*[1], *Ruhi Kapahi*[1], *Mridu Manjari*[2], *Meena Sudan*[3], *Manjit Singh Uppal*[4], *Neeti Rajan Singh*[4]

[1]*Department of Human Genetics, Guru Nanak Dev University, Amritsar, Punjab India, Amritsar, India,* [2]*Department of Pathology, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India, Amritsar, India,* [3]*Department of Radiotherapy, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India, Amritsar, India,* [4]*Department of Surgery, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India, Amritsar, India.*

The objective of this study was to investigate the potential association of haplotypes of six *VEGF* polymorphisms with breast cancer risk in North-West Indians. Samples of 250 breast cancer patients and 250 age and gender matched controls were genotyped for *VEGF* -2578C/A, -2549I/D, -460T/C, +405C/G, -7C/T and +936C/T polymorphisms. Haplotypes were generated to determine the better contribution of *VEGF* polymorphisms to breast cancer risk. Haplotypes CDTCCC (OR = 0.56, 95%CI, 0.38-0.81; p = 0.003) and CDTGCC (OR = 0.63, 95%CI, 0.44-0.92; p = 0.018) of *VEGF* -2578C/A, -2549I/D, -460T/C, +405C/G, -7C/T and +936C/T polymorphisms were significantly associated with decreased risk of breast cancer. CDTCCC haplotype was also significantly associated with reduced risk of breast cancer in pre and post menopausal as well as both obese and non obese patients. Haplotype CDTGCC was marginally associated (p = 0.07) with reduced risk of breast cancer in non-obese patients as compared with non-obese controls where as haplotype AICGTC was marginally associated (p = 0.09) with reduced risk of breast cancer in obese patients when compared with non-obese patients. The CDTGCC haplotype was significantly associated with increased risk of breast cancer in premenopausal obese patients (OR = 1.98, 95%CI, 1.10-3.56; p = 0.02). Our data indicated that CDTCCC and CDTGCC haplotypes of *VEGF* -2578C/A, -2549I/D, -460T/C, +405C/G, -7C/T and +936C/T polymorphisms were significantly associated with breast cancer risk in North-West Indians. Further studies on multiethnic groups with larger sample size are required to confirm our results.

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P12.032.D High rate of pathogenic germline variants in young patients with neuroendocrine tumors

Rachel P. Riechelmann, Mauro D. Donadio, Milton Barros, Karina M. Santiago, Nathalia A. Carvalho, Laura Lopez, Dirce M. Carraro, ***Giovana T. Torrezan***

A.C.Camargo Cancer Center, Sao Paulo, Brazil.

Introduction: Advances in genomics have enabled the recognition of about a hundred cancer predisposing genes (CPG). The contribution of most of these genes to the development of neuroendocrine tumors (NET) is largely unknown. Here, we aim to define the frequency of pathogenic (P) and likely pathogenic (LP) germline variants in known CPGs in young adults with NET.

Methods: We screened germline variants in 100 patients (without known clinical/molecular diagnosis of hereditary cancer syndromes) with lung or digestive NET diagnosed under 45 years. Next generation sequencing was performed using a custom 113 gene panel in an Illumina NextSeq 500. For P/LP variants, amplicon sequencing was performed in tumor DNA to identify loss of heterozygosis (LOH).

Results: Sixteen (16%) patients presented P/LP variants, including genes with established or emerging association to TNE predisposition (*SDHB, MUTYH, CHEK2*) and genes with unknow

relation to TNE risk (*LZTR1, XPC, ERCC2, ERCC3, SLX4*, others). *MUTYH* was the most frequently mutated gene (4 patients: pancreas, midgut, appendix, gastric). The median age and positive family history of cancer were 34 years and 88% for patients with P/LP variants and 35 years and 60% for patients without. LOH was evaluated in 3 tumors so far: two presented absence of LOH for variants in *XPC* and *ERCC2* and one presented LOH for a variant in *SLX4*.

Conclusions: Young adults with NET present a high rate of P/LP variants in CPG, including several DNA repair genes. Tumor analysis can contribute to understanding the role of these variants in TNE development. FAPESP:20018/06269-5

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P12.033.A Cancer Predisposition Syndromes as secondary findings in patients undergoing somatic tumor testing

Alexandra Liebmann[1,2], *Sorin Armenau-Ebinger*[1,2], *Cristiana Roggia*[1,2], *Stephan Ossowski*[1,2], *Andrea Forschner*[3], *Andreas Hartkopf*[4], *Michael Bitzer*[5], *Yvonne Moeller*[6], *Olaf Riess*[1,2], *Christopher Schroeder*[1,2]

[1]*Institute of Medical Genetics and Applied Genomics, University Hospital Tübingen, Tübingen, Germany,* [2]*Centre for Rare Diseases, University Hospital Tübingen, Tübingen, Germany,* [3]*Department of Dermatology, Center for Dermatooncology, University Hospital Tübingen, Tübingen, Germany,* [4]*Department of Obstetrics and Gynecology, University Hospital Tübingen, Tübingen, Germany,* [5]*Department of Internal Medicine 1, University Hospital Tübingen, Tübingen, Germany,* [6]*Center for Personalised Medicine, University Hospital Tübingen, Tübingen, Germany.*

Introduction: Genetic testing for Cancer Predisposition Syndromes (CPS) is currently offered to patients meeting specific clinical criteria such as age of cancer diagnosis or family history. At our Center for Personalised Medicine parallel sequencing of tumor and normal tissue is performed in late-stage cancer patients to identify therapeutic targets. Using blood as normal control allows detecting pathogenic germline variants (PGVs) in CPS-related genes as secondary findings.

Material and Methods: Tumor-normal sequencing was performed in 578 gynecological, skin and gastrointestinal cancer patients without previous genetic diagnosis between 01/2019-01/2021. Next-Generation-Sequencing of a custom cancer panel with more than 700 genes including 41 CPS-related genes (SureSelect XT; Agilent, Germany) was analyzed using an in-house bioinformatics pipeline (megSAP).

Results: In 578 patients 9.7% (n = 56) PGVs in CPS-related genes were identified. PGVs were mainly detected in *ATM* (n = 13), *MUTYH* (n = 10), *CHEK2* (n = 5), *BRCA2* (n = 5). Two patients harbored two different PGVs. Two other cases showed mosaic status in *TP53* and *MSH6*, respectively. One case was suspicious for clonal hematopoiesis in *ATM*. In 36 of the 56 PGVs cases the patient's tumor type matched the associated tumor spectrum. For 10 of the 36 PGVs a second hit in tumor tissue was identified.

Conclusion: This study highlights the utility of parallel tumor-normal sequencing in identifying PGVs causal for CPS in cancer patients who are not necessarily eligible for genetic germline testing. In addition to having impact on clinical management, this approach enables patients and their relatives to optimize individual prevention strategies.

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P12.034.B Subpopulation of cancer stem cells are endowed with distinctive behavior

Letícia A. M. Ferreira, Carlos H. V. Nascimento-Filho, Vilson Serafim-Junior, Márcia M. U. Castanhole-Nunes, Glaucia M. M. Fernandes, Ana L. S. Galbiatti-Dias, Rosa S. Kawasaki-Oyama, Marlon F. Mattos, Maria A. M. S. Bezerra, Érika C. Pavarino, José V. Maniglia, ***Eny M. Goloni-Bertollo***

Faculdade de Medicina de São José do Rio Preto (FAMERP), São José do Rio Preto, Brazil.

Introduction: Cancer stem cells (CSC) are capable of self-renewing and recapitulate the tumor heterogeneity. Due to its resistance to conventional chemotherapy, and association with relapses, this population has been widely investigated. Besides, its association with epithelial to mesenchymal transition requires better comprehension.

Material and methods: Using the Fluorescent Activated Cell Sorting by which we isolated from head and neck cancer subpopulations endowed with stemness properties: $CD44^+$/$CD117^+/133^+$ (CDs^+), and $ALDH^+$ enriched cells, besides their negative control group. Cells sorted were cultivated under DMEM medium, supplemented with 10% FBS, 2% AB/AM, 1% glutamine, and maintained in a 5% CO2-humidified incubator. Total RNA was extracted using a Direct-zol RNA Miniprep Plus isolation kit, following the manufacturer's protocol. Real-time PCR was performed to quantify genes and miRNAs expressions using PCR Master Mix (Life Technologies), with specific probes, and TaqMan miRNA Assay (Thermo Fisher Scientific).

Results: Our findings revealed that downregulation of ALDH mRNA in the CDs^+ population compared with the ALDH enriched population ($^*p < 0.05$). We also observed a fold increase of mRNA expression for VEGFA and ZEB1 in the ALDH enriched population ($^*p < 0.05$ and $^{****}p < 0.0001$). Moreover, the CDs^+ population presents miRNA 200-3p overexpressed, and ZEB 1 inhibited upon transfection what suggests impairing its capacity on undergoing EMT phenotype.

Conclusion: These findings point toward the crucial role of the epigenetic mechanisms, such as microRNAs, in regulating the tumor microenvironment complexity including the levels of stemness and EMT phenotype. Altogether, our study brings new pieces for better understanding the regulation of epigenetic mechanisms and tumor plasticity.

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P12.036.D Introducing Progenetix Beacon- a comprehensive cancer cell line variant knowledge resource

Rahel Paloots*, Michael Baudis*

University of Zürich, Zürich, Switzerland.

Cancer cell lines are good models for studying the disease mechanisms and testing for possible drugs. For the cell lines to be accurate representations of the disease, they would need to be genetically highly similar to their primary neoplasias. Moreover, another issue when working with cell lines is the possible contamination or misidentification of the cell lines. To address both of these concerns, the genetics of both cancer cell lines and their origins will need to be evaluated. Furthermore, cancers as well as cancer cell lines exhibit copy number variation (CNV) profiles that show regions in the chromosomes that have been deleted or amplified. Similarity assessment of these CNV profiles along with examining single nucleotide variants as well, enable the detection of cell lines that are the most accurate representations of the disease. Therefore, we have been building an extensive knowledge resource for cell line copy number and single nucleotide variants (SNVs). The database (Progenetix) contains information about both CNVs and SNVs as well as NCIt and ICDO codes for the disease origin. Additionally, more metadata and other information about cancer cell lines and variants will be available publicly on Progenetix website. The collection of these data enables and facilitates research with cancer cell lines as well as the authentication of the cancer cell lines.

R. Paloots: None. **M. Baudis:** None.

P12.037.A The effect of germline *POLG* gene variants on cervical cancer pathomorphological characteristics and disease outcome

Ieva Golubickaite[1], *Rasa Ugenskiene*[1,2], *Egle Ziliene*[3], *Jurgita Beniusyte*[2], *Arturas Inciura*[2,3], *Elona Juozaityte*[2,3]

[1]*Department of Genetics and Molecular Medicine, Lithuanian University of Health Sciences, Kaunas, Lithuania,* [2]*Institute of Oncology, Lithuanian University of Health Sciences, Kaunas, Lithuania,* [3]*Department of Oncology and Hematology, Hospital of Lithuanian University of Health Sciences, Kaunas, Lithuania.*

Introduction: Cervical cancer ranks as the fourth most common cancer in women worldwide. Despite huge efforts and ongoing research in the field, the effect of germline variants on tumor phenotype and the course of the disease remain unclear. The variations in *POLG* gene, coding for gamma polymerase, involved in mitochondria DNA replication and repair, might be important for carcinogenesis. This study aimed to analyze germline single-nucleotide variants in *POLG* gene and to assess their possible effect on tumor phenotype and disease outcome.

Materials and Methods: A group of 172 cervical cancer patients was analyzed. Tumor pathomorphological parameters and patient data, related to disease outcome, were collected from medical records. Four SNPs (rs3087374, rs2307441, rs2072267, rs976072) in *POLG* gene were selected for the study. Real-time PCR with TaqMan probes was used for the analysis.

Results: *POLG* variant rs3087374 was significantly associated with tumor pathomorphological parameters. Patients with CA genotype and the carriers of A allele had an increased probability of adenocarcinoma histological tumor type, IIIA tumor stage, and pT3 tumors. Additionally, patients with AA genotype in rs2072267 had longer metastasis-free survival than those with GG genotype.

Conclusions: Our data suggest that variations in *POLG* gene might be important in cervical cancer carcinogenesis.

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P12.038.B Targeted analysis of cell-free DNA fragmentation profiles in lung cancer

Anastasia Koval[1], *Daria Rodionova*[1], *Alexey Kazakov*[2], *Konstantin Laktionov*[2], ***Dmitry Shcherbo***[1]

[1]*Institute of Translational Medicine, Pirogov Russian National Research Medical University, Moscow, Russian Federation,* [2]*N.N. Blokhin Cancer Research Medical Center of Oncology, Moscow, Russian Federation.*

Introduction: Accumulating evidence suggests that aberrant genome-wide cell-free DNA (cfDNA) fragmentation patterns reflect the altered epigenetic state of tumor cells in cancer patients and can be applied for cancer diagnostics. Given that cfDNA fragmentation is non-random and fragment lengths tend to be overall shorter in cancer, the cfDNA fragmentation state may differ uneven across the genome in healthy and cancer individuals. We suppose that a measure of cfDNA fragmentation in differentially fragmented regions could serve as a cancer biomarker.

Materials and Methods: We have developed an approach based on a modification of anchored multiplex PCR followed by NGS for deep multiplex fragment lengths profiling that simultaneously targets 25 tumor-specific open-chromatin regions in cfDNA. We tested our approach on 5 samples from patients with lung adenocarcinoma (stages III and IV) and 5 samples from healthy donors.

Results: We have identified 3 regions with the most prominent difference in fragmentation profiles between lung cancer patients and healthy donors that can further be evaluated as novel lung adenocarcinoma markers in cfDNA.

Conclusions: In this proof-of-concept study we demonstrate that cfDNA fragmetnomics may yield a novel class of cancer biomarkers not only at a whole-genome scale but also in a targeted manner. The identification of cancer-specific differentially fragmented regions in larger screening studies may result in an additional dimension of biomarkers complementing liquid biopsy analyses.

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P12.039.C Functional analysis of rare *CHEK2* variants identified in breast cancer patients

Olivia Fuentes Rios[1,2,3], *Marta Santamariña*[2,3,4], *Ángel Vizoso*[5,6], *Belinda Rodríguez*[1,3,4], *Ana Crujeiras*[1,3], *Ana Paula Vega Gliemmo*[1,3,4]

[1]*Instituto de Investigación Sanitaria de Santiago de Compostela, Santiago de Compostela, Spain,* [2]*Universidad de Santiago de Compostela, Santiago de Compostela, Spain,* [3]*Fundación Pública Galega de Medicina Xenómica, Santiago de Compostela, Spain,* [4]*Centro de Investigación en Red de Enfermedades Raras, Madrid, Spain,* [5]*Universidad de A Coruña, A Coruña, Spain,* [6]*Centro de Investigaciones Científicas Avanzadas, A Coruña, Spain.*

Introduction: *CHEK2* (checkpoint kinase 2) germline mutations have been associated with a moderate breast cancer risk. However, for rare missense variants the clinical significance is unknown. CHEK2 protein regulates the cell cycle and is activated in response to DNA damage. Activated CHEK2 phosphorylates proteins and inhibits CDC25C phosphatase, leading to cell cycle arrest and apoptosis. The aim of this study was to analyse, through a kinase activity assay, the functional impact of fourteen *CHEK2* variants identified in female breast cancer patients during the genetic diagnosis.

Materials and Methods: *CHEK2* variants p.Trp114Cys, p.Arg117Gly, p.Ser187Phe, p.Glu239Lys, p.Glu302Lys, p.Met304Val, p.Thr323Pro, p.Ser356Leu, p.Ile364Thr p.Met381Val, p.Ser412Arg,

p.Arg474His, p.Thr476Met and p.Asp488Glu were analysed. Also, *CHEK2* wild-type and *CHEK2*: c.1100delC were used as controls. BL21 *E. coli* were transformed with the wild-type or mutant pDest-566-*CHEK2* vector. The proteins were expressed, purified, and quantified. The kinase activity levels were detected by Immunoblot using an anti-phopho-Cdc25C (Ser216).

Results: We found out that p.Met381Val, p.Ser412Arg and p. Arg474His presented a complete loss of function. Additionally, variants p.Arg117Gly, p.Glu239Lys and p.Ile364Thr showed a partial loss of function, whereas p.Trp114Cys, p.Ser187Phe, p. Glu302Lys, p.Met304Val, p.Thr323Pro, p.Ser356Leu, p.Thr476Met and p.Asp488Glu showed no functional impact. The same result has been previously detected for variants p.Glu239Lys and p. Arg117Gly in a cell-based assay (Wu et al., 2006). In the case of p. Thr476Met variant, contradictory results were previously found, from complete loss to no functional impact.

Conclusions: The information of the functional impact of the analysed fourteen *CHEK2* variants will help to establish their clinical significance in cancer patients.

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P12.040.D Complex karyotype in the course of CLL

*Dorota Koczkodaj[1], Małgorzata Luterek[1], **Zuzanna Dołzycka**[1], Ewa Wąsik-Szczepanek[2], Agata Filip[1]*

[1]Department of Cancer Genetics, Medical University, Lublin, Poland, [2]Department of Hematooncology and Bone Marrow Transplantation, Medical University, Lublin, Poland.

Introduction: Chronic lymphocytic leukemia (CLL) is a lymphoproliferative disease characterized by a heterogeneous clinical course. Clonal chromosome aberrations belong to the most important prognostic and predictive factors in CLL. Complex karyotype (CK) is defined by the presence of ≥3 chromosomal aberrations in cancer cells. The aim of the study was to observe chromosomal abnormalities in CLL with particular attention to CK and their clinical significance in relation to selected prognostic factors.

Methods: The study group comprised 101 consecutive, untreated CLL patients. Cytogenetic banding analysis (CBA) and FISH (fluorescent in situ hybridization) were performed. Expression of CD38 and ZAP70 was assessed by flow cytometry, and the mutation status of *IGVH* and *NOTCH1* was determined by direct sequencing.

Results: CK was detected in 23,8 % of patients by means of CBA. The most frequently detected recurrent changes were deletions of 13q (39,13%), 6q (26,08%), 17p (26,08%) and trisomy 12 (21,74%). Numerical changes and translocations were also observed. A statistically significant relationship was found between overall survival and the occurrence of 17p (del *TP53*) ($p < 0.0056$). CK was found to be a risk factor for the occurrence of disease progression. Neither CD38 and ZAP70 expression nor *IGVH* and *NOTCH1* mutation status were related to CK.

Conclusions: Chromosomal and genomic aberrations are important prognostic factors in CLL. Comparing to FISH, the CBA method captured a broader spectrum of alterations in a larger percentage of patients with CK. These results indicate that CK should be taken into account when making a decision to initiate treatment promptly.

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P12.041.A Exploring clonal evolution and genetic causes of therapy failure in chronic lymphocytic leukemia

***Kristýna Závacká**[1,2], Petr Tauš[2], Karol Pál[2], Kamila Stránská[1,2], Šárka Pavlová[1,2], Anna Panovská[1], Šárka Pospíšilová[1,2,3], Karla Plevová[1,2,3]*

[1]Department of Internal Medicine – Hematology and Oncology, University Hospital Brno & Medical Faculty, Masaryk University, Brno, Czech Republic, [2]Center of Molecular Medicine, Central European Institute of Technology, Masaryk University, Brno, Czech Republic, [3]Institute of Medical Genetics and Genomics, University Hospital Brno & Masaryk University, Brno, Czech Republic.

Introduction: In chronic lymphocytic leukemia (CLL), tumor clones with the mutant *TP53* gene frequently expand in disease relapse of patients treated with chemoimmunotherapy. Such an event often leads to disease course deterioration and therapy resistance. However, there is a small number of patients harboring stable minor *TP53*-mutated clones which do not expand even after several therapy lines. We aimed to identify molecular genetic factors affecting CLL clonal evolution in relation to *TP53* mutation expansions. Revealing the clonal architecture and a mutational profile of leukemic cells may contribute to optimal therapy tailoring.

Materials and Methods: Using whole exome sequencing, we investigated samples from 52 CLL patients with a known clinical course and different scenarios of *TP53* mutation expansions. Our cohort included patients treated with standard chemoimmunotherapy, but also with cell signalling inhibitors.

Results: We identified mutations in genes associated with CLL, such as *SF3B1*, *ATM*, *RPS15*, *MED12*, *NOTCH1*, or *NFKBIE*, but also a large number of non-recurrent mutations, which expanded or diminished differently after specific types of therapy and in relation to *TP53* mutation profiles. We calculated pathway mutation scores and using advanced statistical methods, we defined groups of patients with similar pathway mutation profiles and examined how the groups differed in a clinical course.

Conclusion: Our results aid the understanding of molecular grounds of the clonal evolution in CLL, which is necessary for the rational use of available treatment options and for designing of suitable diagnostic panels. Supported by MUNI/A/1595/2020, MUNI/IGA/1640/2020, AZV NU21-08-00237, and MH-CZ RVO 65269705.

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P12.042.B Search for markers and mechanisms of resistance to TKI therapy

***Elmira P. Adilgereeva**[1], Alex G. Nikitin[2], Diana G. Zheglo[1], Oleg A. Shukhov[3], Svetlana A. Smirnikhina[1], Alexander V. Lavrov[1], Ekaterina Y. Chelysheva[3], Anna G. Turkina[3], Sergey I. Kutsev[1]*

[1]Federal State Budgetary Institution «Research Centre for Medical Genetics», Moscow, Russian Federation, [2]Pulmonology Research Institute, Federal Medical-Biological Agancy of Russia, Moscow, Russian Federation, [3]National Research Center for Hematology, Moscow, Russian Federation.

Chronic myeloid leukemia (CML) is myeloproliferative disease, which is successfully treated with tyrosine kinase inhibitors (TKI), however, 20-40% of patients remain resistant to existing therapy. To identify the prognostic markers of the effectiveness of TKI therapy and mechanisms for the development of resistance, we performed exome and transcriptome sequencing. Blood was sampled from 60 CML patients before starting the TKI therapy. Sequencing was performed on the Illumina NextSeq 550 Sequencing System. Bioinformatic analysis included SnpEff (analysis of all transcripts),

ANNOVAR (analysis of allele frequencies in gnomAD, 1000G, ESP6500, algorithms in silico prediction of pathogenicity of SIFT, PolyPhen2, MutationTaster…), Alamut Batch (influence on splicing, dbsnp, ClinVar, COSMIC, and HGMD). HTSeq (to count the number of reads transcripts), edgeR (count data were analyzed). Pheatmap (R package) was used for the heatmap diagram; only $\log_2$-fold changes with an adjusted *p*-value of 0.10 were considered significant. 33% of patients who are resistant to therapy TKI have loss-of-function variant in the *ASXL1* and *DNMT3A* genes, with 25% only in the *ASXL1* gene, and 8% in *ASXL1* and *DNMT3A*. We have not yet managed to find pathogenetic pathways of resistance, today another 36 samples are at the stage of bioinformatic analysis of the transcriptome. The identified variants in *ASXL1* and *DNMT3A* may be associated with resistance to TKI therapy and serve as prognostic markers of the TKI therapy effectiveness at the stage of CML diagnosis. Possibly, upon completion of the analysis of all transcriptomes, we will be able to find the mechanisms of development of resistance.

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P12.044.D Testing of molecular methods for highlighting rearrangements on BCR-ABL fusion gene

Andra Maria Duca[1], *Andrada Alexiu*[1], *Silvia Aposteanu*[2], *Mihaela Dragomir*[2], *Danut Cimponeriu*[1], *Ileana Stoica*[1]

[1]*Department of Genetics, University of Bucharest, Bucharest, Romania,* [2]*Department of Hematology - Molecular Biology, Clinical Institute, Bucharest, Romania.*

Introduction: Testing of translocation between chromosome 9 (Abelson murine V gene leukemia viral oncogene homolog1-ABL1) and 22 (Breakpoint Cluster Region-BCR) and mutations in the ABL kinase domain are useful for diagnostic of chronic myeloid leukemia (CML) and guide the treatment. Wet tested the ability of molecular methods to identify E255K/V and T315I mutations in BCR-ABL gene.

Materials and methods: Blood samples from twenty CML patients with Ph^+ chromosome (55 ± 17 years old) was used for messenger RNA extraction. The cDNA was amplified by nested PCR and the amplicons were digested with MnlI and DdeI endonucleases. Restriction fragments were resolved by polyacrylamide gel electrophoresis to highlight the presence of the E255K/V and the T315I mutations in BCR-ABL fusion gene.

Results: This method revealed the E255K/V and T315I mutations in the BCR-ABL gene in two samples. The male with T315I mutation has 79 years old and had been under the therapy for 11 years. The patient responded to treatment after the identification of mutation. The E255K/V mutation was detected in a male who died shortly after inclusion in this study. It was observed that patients with CML who had acquired mutations did not respond to imatinib therapy and required customization. The frequency of mutation is estimated to be in accordance with the values reported for other populations.

Conclusions: This method based on reverse transcription and nested PCR amplification of the fusion gene BCR-ABL is easy to implement, is relatively rapid and inexpensive as compared with other techniques to identify the mutations.

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P12.047.C Detection of T315I in patients with Chronic Myeloid Leukemia by ddPCR preliminary results

Hristo Y. Ivanov[1], *Aleksandar Linev*[1], *Ivan Zheliazkov*[1], *Veselina Goranova-Marinova*[2], *Zhanet Grudeva-Popova*[3], *Vili Stoyanova*[1]

[1]*Medical University Plovdiv, Department of Pediatrics and Medical Genetics, Plovdiv, Bulgaria,* [2]*Medical University Plovdiv, First Department of Internal Medicine, Clinic of Hematology, Plovdiv, Bulgaria,* [3]*Medical University Plovdiv, Department of Clinical Oncology, Plovdiv, Bulgaria.*

Introduction: Chronic myeloid leukemia is oncohematological disease, characterized by a BCR-ABL1 fusion protein. Through treatment patients may acquire mutations in the kinase domain of *ABL1*. The T315I mutation is key mutation with relevant clinical implications - can only be overcome by third generation tyrosine kinase inhibitors. The aim of our study is to detect T315I mutation in patients with CML by ddPCR and evaluate if detection of small clones could support an early clinical decision to change of treatment.

Materials and methods: The study included 17 patients with CML. gDNA was extracted from whole blood and the samples were tested for T315I by ddPCR.

Results: T315I was detected in 2 patients, in one of them the variant allele frequency was 29% in the other it was a small clone mutation - 0.1%.

Discussion: The use of ddPCR to screen for specific, highly relevant mutations as T315I that confer resistance to treatment holds great promise. Our study suggests that ddPCR can also be an effective and sensitive method for the detection of the T315I mutation in the setting of CML. The aim of mutation testing is to identify those who need a change in therapy rather than a "watch and wait" approach, since detection of a TKI-resistant mutation is an indication for therapeutic switch. The continuation of this study will be directed toward exploring the clinical advantage of the greater sensitivity offered by ddPCR, and the assessment how to best place ddPCR in the management of patients without an optimal response.

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P12.048.D Low mitochondrial DNA copy number correlates with longer progression free survival in clear cell renal cell carcinomas

Sarah BELLAL, *Cyrielle Rolley, Jeremy Richard, Léo Bove, Vincent Lecorre, Marie - Christine Rousselet, Odile Blanchet, Pierre Bigot, Vincent Procaccio, Céline Bris*

CHU Angers, Angers, France.

Clear cell renal cell carcinoma (CCRCC) is the most common renal cancer whose prognosis is currently assessed by imperfect clinical scores. It is characterized by a metabolic reprogramming suggesting a major role of mitochondria in tumor development. Mitochondrial DNA (mtDNA), present in thousands of copies/cell, encodes essential polypeptides required for oxidative phosphorylation. MtDNA copy number (mtDNAcn) variations and mtDNA mutations have been associated with various clinical outcomes in cancer. This retrospective study evaluates the influence of mtDNA genetics on the prognosis in patients with CCRCC.Twenty-one patients following for CCRCC, including four metastatic, were included after informed consent. Clinical and survival data were collected for each patient. DNA extraction, mtDNA sequencing and mtDNAcn were performed on tumors and matched adjacent-normal tissues. Patient's mtDNAcn variation between tumor and adjacent-normal tissue was expressed as ratio, and cohort subdivided into two groups according to the median. Progression

free survival (PFS) was significantly longer in the low than in the high mtDNAcn ratio group: 1137 days vs 252 days (p < 0,001*). On the other hand, no correlation between PFS and somatic variants, age at diagnosis, CCRCC grading, or lymphovascular invasion was found. NGS of the whole mtDNA did not reveal deletions or common variants in tumor samples. Interestingly, few patients accumulated somatic mtDNA variants with different heteroplasmy levels between the tumor and metastasis potentially important for tumor aggressiveness. Hence, mtDNAcn appears to be a promising independent prognostic factor in CCRCC.This work received grants from the University and Hospital of Angers and Ligue contre le cancer.

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P12.049.A Analysis of genomic complexity in patients with chronic lymphocytic leukemia (CLL) using optical genome mapping

Anna Puiggros[1,2], *Silvia Ramos-Campoy*[1,2], *Tuomo Mantere*[3], *Marta Salido*[1,2], *Carme Melero*[1,2], *María Rodríguez-Rivera*[1,2], *Sandrine Bougeon*[4], *Rosa Collado*[5], *Mireia de la Rosa*[1,2], *Eva Gimeno*[6,7], *Rocío García-Serra*[5,8], *Sara Alonso*[9], *Marco Moro*[9], *María Dolores García-Malo*[10], *Jacqueline Schoumans*[4], *Alexander Hoischen*[3,11], *Blanca Espinet*[1,2]

[1]*Molecular Cytogenetics Laboratory, Pathology Department, Hospital del Mar, Barcelona, Spain,* [2]*Translational Research on Hematological Neoplasms Group, Cancer Research Program, Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Barcelona, Spain,* [3]*Department of Human Genetics, Radboud University Medical Center, Nijmegen, Netherlands,* [4]*Oncogenomic laboratory, Hematology service, Lausanne University Hospital, Lausanne, Switzerland,* [5]*Department of Hematology, Consorcio Hospital General Universitario, Valencia, Spain,* [6]*Department of Hematology, Hospital del Mar, Barcelona, Spain,* [7]*Applied Clinical Research in Hematological Malignances, Cancer Research Program, Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Barcelona, Spain,* [8]*Research Foundation from Hospital General Universitario, Valencia, Spain,* [9]*Department of Hematology, Hospital Universitario Central de Asturias, Oviedo, Spain,* [10]*10 Department of Hematology, Hospital Universitario Morales Meseguer, Murcia, Spain,* [11]*Radboud University Medical Center Center for Infectious Diseases (RCI), Department of Internal Medicine and Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, Netherlands.*

Background: Complex karyotype (CK) predicts poor prognosis in chronic lymphocytic leukemia (CLL). Although CK detection is routinely assessed by chromosome banding analysis (CBA) or chromosomal microarrays (CMA), the obtained results are not equivalent. Optical genome mapping (OGM) is a novel technology that overcomes most of the limitations of CBA/CMA, and would potentially replace them in the near future. We aimed to analyze the utility of OGM in the cytogenetic assessment of CLL patients.

Methods: Tumor DNA from 22 CLL patients were analyzed by OGM (Bionano Genomics), 12 of them carried a CK and 10 were non-CK. OGM results were compared with available CBA, FISH and CMA (ThermoFisher) data.

Results: OGM detection rate of known abnormalities was 93.8% (15/16) and 87.2% (109/125) in non-CK and CK group, respectively. Size and coordinates of copy number alterations detected by OGM and CMA were highly concordant. OGM allowed the interpretation of complex rearrangements or provided additional structural information to CMA in 11/22 (50%) patients. All cases showed several additional abnormalities of unknown clinical significance by OGM. Globally, more complex genomic profiles were identified in the CK group.

Conclusions: 1. OGM is a valuable tool to assess genomic complexity in CLL that effectively detects the vast majority of the abnormalities usually defined by a combination of standard methods in a single assay; 2. Several additional abnormalities of unknown clinical significance are identified by OGM; 3. Further studies are required to define criteria for OGM genomic complexity with clinical significance in CLL.

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P12.050.B Highly-sensitive approach for characterizing microsatellite instability in normal tissue and tumors from biallelic germline mismatch repair mutation carriers

*Fátima Marín**[1], *Júlia Canet**[1], *Vanesa Pérez-Alonso*[2], *Sandra Tapial*[3], *Iciar Rey*[3], *Nuria Carrizo-Mijarra*[4], *Yolanda Rodriguez-Gil*[4], *Gabriel Capellá*[1], *Luis Ignacio González-Granado**[5], ***Marta Pineda****[1], *Daniel Rueda**[3]

[1]*Hereditary Cancer Program, Catalan Institute of Oncology, Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), ONCOBELL Program, CIBERONC, L'Hospitalet de LLobregat, Spain,* [2]*Pediatrics Department, Doce de Octubre University Hospital, i+12 Research Institute, Madrid, Spain,* [3]*Hereditary Cancer Laboratory, Doce de Octubre University Hospital, i+12 Research Institute, Madrid, Spain,* [4]*Department of Pathology, Doce de Octubre University Hospital, i+12 Research Institute, Madrid, Spain,* [5]*Primary Immunodeficiencies Unit, Pediatrics, University Hospital 12 Octubre, Madrid, Spain.*

Constitutional MisMatch Repair Deficiency (CMMRD) is a rare and devastating childhood-onset cancer predisposition syndrome caused by biallelic mutations in MisMatch Repair (MMR) genes. Microsatellite instability (MSI), a molecular hallmark of MMR-deficiency, is not always detected in tumors from CMMRD carriers by conventional MSI analysis methods. Recently, novel highly-sensitive NGS approaches have allowed the detection of low-level MSI in blood samples from CMMRD individuals (PMID 31494577 and 30740824). Our aim was to better characterize MSI metrics in normal-tumor samples from CMMRD patients using a highly sensitive approach. Blood, normal and tumor tissue DNA samples from three CMMRD patients were analyzed using a recently developed high-sensitivity MSI approach. Two metrics, MSI score (percentage of unstable markers) and mean frequency of unstable markers (sum of frequencies of all allele lengths different from the wildtype), were calculated and compared with blood control samples and sporadic MSI tumors. All tumors (Wilms tumors, glioblastoma and lymphomas, n = 8) and non-neoplastic samples (n = 19) from the three CMMRD patients showed a positive hs-MSI score (>4.576). CMMRD tumors showed significantly higher mean instability frequency than non-neoplastic tissues, with no overlapping (p = 1.849e-06). A high correlation between unstable markers was observed in blood samples and lymphomas.. Of note, MSI score and mean frequency of unstable markers was significantly lower in CMMRD tumors when compared to sporadic MSI tumors (p = 0.002). The hs-MSI tool detected MSI in non-neoplastic tissues from CMMRD individuals regardless the tissue origin. Our approach may also be useful in the characterization of CMMRD-associated tumors.

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P12.051.C Multiplexed high-throughput MSREqPCR qualification of Colon Cancer DNA-methylation biomarkers in plasma cfDNA

Andreas Weinhaeusel[1], *Walter Pulverer*[1], *Silvia Schönthaler*[1], *Jasmin Huber*[1], *Kristi Kruusmaa*[2], *Bhangu Jagdeep Singh*[3], *Klemens Vierlinger*[1]

[1]*AIT, Vienna, Austria,* [2]*Universal Diagnostics S.L., Sevilla, Spain,* [3]*Medical University of Vienna, Vienna, Austria.*

Background: There is great need to improve colorectal cancer (CRC) early diagnosis, treatment stratification and therapy monitoring. Methylation-sensitive restriction enzyme coupled qPCR (MSREqPCR) has been setup to test the diagnostic performance in plasma cell-free DNA (cfDNA) of candidate markers deduced from CRC tissue.

Methods: We setup MSREqPCR assays for DNA methylation analysis using cfDNA isolated from plasma. 48 candidate methylation markers were evaluated in 88 cfDNAs (44 CRC, 44 controls). The workflow was then used to perform 500plex analysis on 770 plasma cfDNA samples, selecting markers to define robust classifiers.

Results: A panel of 48 candidate DNA-methylation-markers selected from tissue DNA testing by targeted microarray-analysis, was used to for bisulfite-qMSP confirmation giving 96-100% correct classification. Testing the 48 markers in plasma cfDNA by MSREqPCR (44 CRC, 44 controls) provided in ROC analysis an AUC>0.85 for WT1, PENK, SPARC, GDNF, TMEFF2, and DCC. Applying the 48-plex-panel to liquid biopsies from progressed CRC a 3-gene-signature (BOLL, DCC, SFRP2) was defined supporting patient stratification and therapy monitoring. Applying our workflow for testing 180 markers on 1000 cfDNA samples enabled definition of a robust and highly reliable diagnostic methylation 12-plex classifier of AUC 0.88.

Conclusions: MSREqPCR allows the targeted investigation of 96 DNA methylation sites using the cfDNA content of only 2 ml of plasma. Performance of methylation markers in tissue and in liquid biopsy as well as in different clinical settings differ largely. MSREqPCR is best suited for efficient selection of markers and directly applicable for PCR based non-invasive testing.

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P12.052.D Concordant molecular profiles between metachronous colorectal cancers: a colonic or rectal metastasis?

Jose Perea[1,2], *Luis Corchete*[3,4,5], *Andreana N. Holowatyj*[6,7], *Juan L. Garcia*[3,4], *Sandra Tapial*[8], *Yasuyuki Okada*[9], *Ajay Goel*[10], *Damian García-Olmo*[1,2], *Miguel Urioste*[11], *Rogelio González-Sarmiento*[3]

[1]*Fundación Jiménez Díaz University Hospital, Madrid, Spain,* [2]*Fundación Jiménez Díaz University Hospital Health Research Institute, Madrid, Spain,* [3]*Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain,* [4]*Hematology Department, University Hospital of Salamanca. Cancer Research Center (CiC-IBMCC, CSIC/USAL), Salamanca, Spain,* [5]*Center for Biomedical Research in Network of Cancer (CIBERONC), Salamanca, Spain,* [6]*Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, USA,* [7]*Vanderbilt-Ingram Cancer Center, Nashville, TN, USA,* [8]*12 de Octubre Research Institute., MADRID, Spain,* [9]*Department of Gastroenterology and Oncology. Tokushima University Graduate School of Biomedical Sciences., Tokushima, Japan,* [10]*Department of Molecular Diagnostics and Experimental Therapeutics, Beckman Research Institute of City of Hope. City of Hope Comprehensive Cancer Center, Monrovia, CA, USA,* [11]*Clinical Cancer Unit. Human Genetics Group, Human Cancer Genetics Program, Spanish National Cancer Centre (CNIO), Madrid, Spain.*

Introduction: Some multiple primary colorectal cancers (CRCs) (synchronous or metachronous) may derive from a similar clonal origin. The most frequent etiology is a CRC predisposition, but other causes could lead to the question of whether multiple primary CRCs arise from one primary tumor and a metastatic lesion in the colorectum.

Material and Methods: Using a cohort of 48 cases diagnosed with Metachronous CRCs (excluding hereditary cases), we characterized microsatellite instability (MSI) and performed Next Generation Sequencing, with a 50-gene panel, to define the concordant cases (same MSI profile and identical pathogenic mutations between both paired-tumors). We also carried out genome-wide DNA methylation analysis. To confirm concordance of CRC paired samples, we achieved unsupervised multidimensional scaling (MDS) of the methylation profiles, with both concordant and discordant cases.

Results: A total of 4% of cases (2 of 48) had concordant microsatellite stability and pathogenic mutations in both tumors (one with identical *TP53* mutation in both; the second with *TP53* and *BRAF* mutations). Both cases of concordant CRCs had the first tumor in the right colon and the metachronous malignancy in the rectum. One case was initially diagnosed with stage IV CRC; the second developed metastatic disease 2 years post-metachronous CRC. MDS analysis revealed that concordant cases were grouped together via similar methylation profiles.

Conclusions: The potential that a proportion of multiple primary CRSs may be composed of a primary CRC and a metastatic lesion with concordant molecular profiles opens a clinically relevant avenue in diagnostic and therapeutic management of this CRC subtype.

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P12.053.A Omic data integration in the search of novel candidate genes of susceptibility to colorectal cancer

Anael Lopez-Novo[1], *Juan Fernandez-Tajes*[1], *Jorge Amigo*[1], *Andres Dacal*[2], *David Remedios*[3], *Joaquin Cubiella*[3], *Victoria Alvarez-Sanchez*[4], *Maria Jesus Ladra-Gonzalez*[5], *Fernando Fernandez-Lopez*[5], *Ana Alvarez-Castro*[5], *Jose Manuel Cameselle-Teijeiro*[6], *Miriam Cuatrecasas*[7], *Francesc Balaguer*[8], *Sergi Castellvi-Bel*[8], *Ceres Fernandez-Rozadilla*[1], *Clara Ruiz-Ponte*[1,9]

[1]*Fundacion Publica Galega de Medicina Xenomica, Instituto de Investigacion Sanitaria de Santiago (IDIS), Grupo de Medicina Xenomica-Universidade de Santiago de Compostela, Santiago de Compostela, Spain,* [2]*Servicio de Digestivo, Hospital Universitario Lucus Augusti, Instituto de Investigacion Sanitaria de Santiago de Compostela (IDIS), Lugo, Spain,* [3]*Servicio de Digestivo, Complexo Hospitalario Universitario de Ourense, Centro de Investigacion Biomedica en Red de Enfermedades Hepaticas y Digestivas (CIBERehd), Ourense, Spain,* [4]*Servicio de Digestivo, Complexo Hospitalario de Pontevedra, Pontevedra, Spain,* [5]*Servicio de*

Digestivo, Complexo Hospitalario Universitario de Santiago de Compostela, Santiago de Compostela, Spain, [6]Servicio de Anatomia Patologica, Complexo Hospitalario Universitario de Santiago de Compostela, Santiago de Compostela, Spain, [7]Servicio de Anatomia Patologica, Institut D'Investigacions Biomediques August Pi i Sunyer, Centro de Investigacion Biomedica en Red de Enfermedades Hepaticas y Digestivas (CIBERehd) and Tumor Bank-Biobank, Hospital Clinic, Barcelona, Spain, [8]Gastroenterology Department, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD), Hospital Clínic, Universitat de Barcelona, Barcelona, Spain, [9]Centro de Investigacion Biomedica en Red de Enfermedades Raras (CIBERER), Santiago de Compostela, Spain.

Introduction: In recent years the main strategy to explain the missing heritability in colorectal cancer (CRC) relied on whole-exome sequencing (WES) analysis. However, this strategy was not as successful as expected, possibly due to the genetic heterogeneity underlying CRC. Our aim is to molecularly characterize early-onset MMR-proficient CRC at somatic level to further identify novel candidate susceptibility genes by integrating germline and tumor omic data.

Materials and Methods: WES, RNA-seq and methylation array were performed on paired normal-tumor tissue from a phenotypically homogeneous cohort of 20 early-onset (<50y) MMR-proficient CRC patients. Germline and somatic variant calling (HaplotypeCaller and Mutect2, respectively), signature profile (SigProfiler), consensus molecular subtyping (CMSCaller), differential expression analysis (DESeq2) and methylation profile were carried out.

Results: Colorectal tumors were molecularly subtyping as: CMS1 (10%), CMS2 (35%), CMS3 (25%), CMS4 (10%) and unknown (20%). Filtering germline exome data according to the over-represented pathways, found in each subgroup, and also tumor signatures did not identify recurrent rare variation within the different groups. Currently, we are conducting a joint analysis using data from WES, RNA-seq and methylation array, to achieve a more accurate molecular classification and a more reliable germline filtering strategy.

Conclusions: These data show the molecular heterogeneity underling CRC in a phenotypically homogeneous cohort. The integration of somatic and germline omics will allow a more comprehensive prioritization of germline variants, increasing the probabilities to identify new CRC predisposition genes. As a result, a more personalized genetic diagnostic could be achieved.Grant support: ISCIII and FEDER funds PI17/00509; Predoctoral Fellowship (Xunta de Galicia)

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P12.054.B Contribution of Pathogenic Variants in Genes Predisposing to Colorectal Cancer by Pan-Cancer Panel Testing

Frida Eiengård[1,2], *Anna Rohlin*[1,2], *Torbjörn Olausson*[1,2], *Ulf Lundstam*[3], *Margareta Nordling*[1,2]

[1]*Department of Laboratory Medicine, Institute of Biomedicine, Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden,* [2]*Department of Clinical Genetics and Genomics, Sahlgrenska University Hospital, Gothenburg, Sweden,* [3]*Department of Surgery, Sahlgrenska Academy at University of Gothenburg, Sahlgrenska University Hospital/Östra, Gothenburg, Sweden.*

Introduction: The number of pathogenic variants in colorectal cancer (CRC) predisposing genes is continuously increasing. Interpretation of variants involves classifications and association to CRC phenotypes. We are gradually improving the genotype to phenotype correlation between the genes and the CRC syndromes with the aim to improve recommendations for surveillance. However, we do not know much about the contribution of pathogenic variants in CRC genes to other hereditary cancer syndromes. We present results from analysing variants in 328 genes including CRC predisposing genes in patients referred for suspicion of other rare hereditary cancers (not CRC or breast/ovarian cancer).

Materials and methods: The study included 68 patients with suspected hereditary cancer (renal cancer, neuroendocrine cancer, melanoma and neurofibromatosis) referred to the Cancer Genetics Counselling Clinic at Sahlgrenska University Hospital, Gothenburg, Sweden. Variant screening was performed using a whole-genome sequencing (WGS) approach. Variants were evaluated regarding pathogenicity using bioinformatic filtration and manual variant classification.

Results: Pathogenic variants and variants of uncertain clinical significance were identified in a number of the CRC associated genes in the patients.

Conclusions: Extended panels might be of value to catch variants in patients with suspicion of hereditary cancer in general. Surveillance guidelines in CRC might in some cases need to be adjusted to also include screening for tumours related to other rare cancer syndromes. The study was supported by grants from the Swedish state under the agreement between the Swedish government and the county councils, the ALF-agreement (ALF-725011) and the Swedish Cancer Society (Grant no. 19 0351).

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P12.055.C Validation of liquid biopsy hotspot assays for assessing recurrence and progression in colorectal cancer patients

Ariane Hallermayr[1,2,3], *Verena Steinke-Lange*[1,4], *Anna Benet-Pagès*[1], *Markus Rentsch*[5,6], *Holger Vogelsang*[7], *Maike De Wit*[8,9], *Christopher Haberl*[10], *Elke Holinski-Feder*[1,4], *Julia M. A. Pickl*[1,4]

[1]*MGZ - Medizinisch Genetisches Zentrum, Munich, Germany,* [2]*Pettenkofer School of Public Health, Munich, Germany,* [3]*Institute for Medical Information Processing, Biometry, and Epidemiology –IBE, LMU Munich, Munich, Germany,* [4]*Medizinische Klinik und Poliklinik IV, Campus Innenstadt, Klinikum der Universität München, Munich, Germany,* [5]*Department of General, Visceral and Thorax Surgery, Klinikum Ingolstadt, Ingolstadt, Germany,* [6]*Department of General, Visceral, Vascular and Transplant Surgery, University Hospital Munich, Ludwig-Maximilians University of Munich, Campus Großhadern, Munich, Germany,* [7]*Department of General, Visceral, Thoracic and Endocrine Surgery, Klinikum Garmisch-Partenkirchen, Teaching Hospital, Ludwig Maximilian University Munich, Garmisch-Partenkirchen, Germany,* [8]*Department of Hematology and Oncology, Vivantes Klinikum Neukoelln, Berlin, Germany,* [9]*Department of Oncology, Vivantes Auguste-Viktoria-Klinikum, Berlin, Germany,* [10]*Department of Oncology and Hematology, Barmherzige Brüder, Klinikum St. Elisabeth, Straubing, Germany.*

Introduction: The BloodPac Consortium recently released guidelines for analytical validation of NGS-based liquid biopsy assays for diagnostic testing. We extend these protocols to more sensitive

droplet digital PCR (ddPCR) assays, and provide clinical validation for determination of disease recurrence and progression.

Material and Methods: Mutant variant allele frequencies (VAFs) in plasma were measured by *BRAF* p.V600E and *KRAS* p.G12/p.G13 ddPCR assays in 22 colorectal cancer (CRC) patients, from whom clinical data were available.

Results: Analytical validation of *BRAF* p.V600E and *KRAS* p.G12/p.G13 assays revealed a cutoff for residual disease detection of 0.02% and 0.11% VAF and accurate quantification for ≥0.52% and ≥0.41% VAF in plasma, respectively. Clinical validity of ddPCR assays for residual disease detection was confirmed in 19 samples of R0 resected CRC patients, as ctDNA detection was in line with clinical evidence: In 5/19 patients, residual disease was detected. In 4 of these 5 patients, metastases were confirmed. In 14/19 patients no residual disease was detected. Only 1 of these 14 patients developed metastases. Further, association of ctDNA detection rates with tumor stages was shown, as 0/4 stage-I, 4/8 stage-II, 3/4 stage-III and 6/6 stage-IV patients were found ctDNA-positive. Only newly diagnosed patients were tested to prevent ctDNA perturbation due to surgery or treatment interventions. Clinical validity of ddPCR assays for treatment monitoring was shown, as ctDNA quantities were associated with response or resistance to chemotherapy in 7/7 patients.

Conclusions: We proof analytical and clinical validity of our liquid biopsy assays to determine recurrence and progression in CRC.

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P12.056.D Assessing the effectiveness of current UK guidelines on familial colorectal cancer (CRC) risk

Kyriaki Christou[1], *Jonathan N. Berg*[2], *Jacqueline Dunlop*[2]

[1]*University of Dundee, Dundee, United Kingdom,* [2]*Department of Clinical Genetics, Ninewells hospital, Dundee, United Kingdom.*

Introduction: Family history (FH) of CRC is a frequent reason for referral to Clinical Genetics in the UK. The British Society of Gastroenterologists (BSG) guideline stratifies patients to risk categories (low/population, low-moderate, high-moderate and high) according to FH and known penetrant mutations. We investigated how effectively BSG guidelines categorise people at increased risk of CRC.

Methods: FH data was obtained for all unaffected people with a family history of CRC, referred to Tayside clinical genetics from 2000-2009. Risk category according to BSG guidance was assigned *de novo*. Individuals who went on to develop adenomatous polyps or CRC were identified by record linkage.

Results: 1120 patients were identified and after exclusion criteria, there were 728 non-polyposis patients (288 low-risk, 316 moderate-risk and 121 high-risk, including 31 mutation carriers). 8 invasive CRC developed, 2 in low, 3 in moderate and 3 in high-risk groups. There was a significant increased risk of CRC development in mutation carriers. There was no significant difference in the rate of CRC development between the risk-groups. There was a significantly higher risk of polyp detection in all categories compared to the low-risk group.

Conclusions: The mutation group have a significantly higher risk of CRC development, but regular screening appears to reduce this risk. Colonoscopic surveillance appears effective in reducing the cancer incidence in moderate and high-risk groups, presumably through polyp removal, thereby supporting continued use of the current guidance. In this context, BSG guidelines appear to effectively stratify risk for familial CRC.

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P12.057.A Application of targeted next-generation sequencing in primary and metastatic colorectal cancer using hotspot panel for detection of potentially therapeutically relevant rare variants

Zora Lasabová[1], *Peter Mikolajcik*[2], *Dusan Loderer*[3], *Marian Grendar*[3], *Eva Gabonova*[2], *Ivana Kasubova*[4], *Tatiana Burjanivova*[1], *Michal Kalman*[5], *Lukas Plank*[6], *Ludovit Laca*[2]

[1]*Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Dept. of Molecular Biology and Genomics, Martin, Slovakia,* [2]*Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Clinic of Surgery and Transplant Center, Martin, Slovakia,* [3]*Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Biomedical Center Martin, Martin, Slovakia,* [4]*Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Biomedical Center Martin, Martin, Slovakia,* [5]*Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Dept. of Pathological Anatomy, Martin, Slovakia,* [6]*Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Dept. of Pathological Anatomy, Martin, Slovakia.*

Introduction: In the practice, for the treatment of metastatic colorectal cancer (mCRC) patients usually first generation methods can be applied to detect therapeutically relevant variants. The implementation of NGS allows to test that patient´s biopsy also for variants for which drugs are yet under development, or for rare variants still allowing the patients to benefit from personalized therapy.

Methods: We used the Ampliseq Cancer HotSpot multigene panel with 2800 hot spots in 50 genes, and analyzed 87 tissue samples from 55 patients with primary CRC (pCRC) and 31 mCRC.

Results: In total, we identified 164 somatic variants, which were classified as pathogenic. We identified mutations in *TP53* (47%), and the *RAS* family (46%) genes, of which *KRAS* and *NRAS* represented 39% and 7%, respectively, followed by *APC* (37%), and *PIK3CA* in 12% of samples. In 30 samples, two driver mutations were present in one sample, and we did not find any of mutations present in our panel in 9 patients. In one patient, we identified a potentially therapeutically significant combination of the *KRAS* G12A and *BRAF* D594E. Another patient had a potentially sensitive mutation to anti-EGFR therapy *KRAS* A59T along with the *PIK3CA* mutation E545K. In two liver metastases from one patient, we identified two different mutations *KRAS* G12C and *NRAS* Q61K together with the *PIK3CA* mutation E545K in both metastases.

Conclusion: Our results may have potential significance for molecular tumor boards in personalized tumor therapy decisions. Supported by the APVV-16-0066 and ITMS 313011V446 projects, funded by the EU resources.

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P12.058.B The interplay between regulatory variants, transposable elements and gene expression in the context of colorectal cancer

Nikolaos M. R. Lykoskoufis[1], *Halit Ongen*[1], *Evarist Planet*[2], *Didier Trono*[2], *Emmanouil T. Dermitzakis*[1]

[1]Department of Genetic Medecine and Development, University of Geneva, Geneva, Switzerland, [2]School of Life Sciences, Ecole Polytechnique Federale de Lausanne (EPFL), Lausanne, Switzerland.

There is increasing evidence of transposable element (TEs) activation during tumorigenesis. However, it still remains unknown whether TE expression is under genetic control and what is the causal relationship between eQTLs, TEs and genes. To address that, we performed *cis*-eQTL analysis using genotypes and mRNA-seq data for 275 normal and 276 tumor samples from the SYSCOL colorectal cancer (CRC) cohort. At 5% FDR, we discovered 10'111 and 5'152 TE-eQTLs as well as 6'856 and 1'539 gene-eQTLs in normal and tumor samples, respectively. We observed more independent eQTLs per gene than per TEs in either state and a smaller distance of eQTLs to the TSS of TEs compared to genes ($P < 0.001$ in normal and tumor). Of the identified TE-eQTLs, 376 are tumor-specific and display differences in methylation patterns (Wilcoxon $P = 0.017$) and enrichment for binding of transcription factors (LoVo cell line ChIP-seq data) compared to the 524 discovered shared TE-eQTLs. To assess the causal relationship between eQTLs, TEs and genes, we used Bayesian networks on 12'379 normal and 9'714 tumor eQTL-TE-gene triplets identified by association testing. We observed an increase of TEs being causal for gene expression changes in tumor compared to normal (Fisher $P = 6.34e\text{-}11$) and identified 79 triplets where TEs become causal for changes in gene expression for 51 cancer driver genes (5 CRC-specific) in tumor making these TEs potential drivers of cancer driver genes. This study highlights a substantial role for TEs as drivers of gene expression and sets a framework to better understand TE effects in cancer.

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P12.059.C The combination of ruxolitinib and MK2206 inhibits cell growth via activating P53 and PTEN expression and decreasing PI3K expression in triple negative breast cancer cell line

Esin Guvenir Celik[1], *Onur EROGLU*[1,2]

[1]Department of Molecular Biology and Genetics, Bilecik Seyh Edebali University, Bilecik, Turkey, [2]Biotechnology Research and Application Center, Bilecik Seyh Edebali University, Bilecik, Turkey.

Introduction: Triple-negative breast cancer(TNBC) is the most aggressive and carries the poorest prognosis, largest recurrence and lowest survival rate. A tumor-specific receptor or pathway-related target for TNBCs has not yet been developed. Ruxolitinib is an orally available receptor tyrosine kinase inhibitor that targets JAK1 and JAK2. The aim of this study; firstly to decrease the side effects of ruxolitinib by combined treatment with selective Akt inhibitor MK-2206 and secondly to investigate the effect of combined treatment on PI3K/AKT signaling pathway in MDA-MB-231.

Materials and Methods: Cells were cultured. Drug doses were determined by MTT assay. A colony formation assay was performed to determine the metastatic effect of drugs on cells. Protein was isolated from treated-untreated cells. Expression levels of P53, PTEN, PI3K and AKT genes were determined by western blotting. Western blot results were analyzed to get band intensities by Image J program.

Results: The cell viability is 50.1% for 22.5μM Ruxolitinib, 55.91% for 7.5μM MK-2206, 54.99% for 18μM Ruxolitinib+5μM MK-2206. The combination of Ruxolitinib and MK-2206 significantly reduced colony formation compared with the control group. We observed that combined treatment activates PTEN and P53, decreases PI3K expression in MDA-MB-231 cells.

Conclusions: Our study indicates for the first time that inhibition of PI3K/AKT signaling with combined treatment of the selective AKT inhibitor MK-2206 and JAK1/2 inhibitor Ruxolitinib and reduces colony formation with combined treatment in MDA-MB-231 cells. This study was financially supported by Bilecik Seyh Edebali University Research Projects (Project No. 2018-02.BSEÜ.01-01).

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P12.060.D A comprehensive genomic profiling (CGP) approach for somatic and germline mutation detection purpose in FFPE cancer samples

Francesco Orsini[1], ***Anna Dellai***[1], *Matteo Giacomin*[2], *Lara Walczer Baldinazzo*[1], *Fabiana Crò*[1], *Cristina Lapucci*[1], *Marcello Gambacorta*[1]

[1]Synlab Italia, Castenedolo, Italy, [2]University of Perugia, Perugia, Italy.

Introduction: CGP is one of the most recent approach in tumor mutation analysis. Many laboratories are focused in mutational screening by classical molecular techniques; CGP detects genomic aberrations and is increasingly being utilized to match patients to targeted therapies against oncogenic drivers. CGP also detects germline variants and might be useful for patient's management.

Materials and Methods: we applied a CGP approach for mutational screening, Tumor Mutation Burden calculation, Microsatellite Instability and to identify RNA rearrangments, RNA rearrangements detection. DNA and RNA from 42 FFPE tumor samples (melanoma, colorectal cancer, breast cancer and prostatic cancer) were processed using TSO500 and sequencing was performed on NextSeq 550Dx. We applied two bioinformatic pipelines for variants filtering. Germline variants were confirmed analyzing non tumor tissue when available.

Results: Somatic variants were classified in 4 TIERS following AMP/ASCO/CAP guidelines. 14 TIER-IA variants were confirmed by gold standard method. TMB and MSI were determined and MSI-H status was confirmed by an IVD kit. Germline variants were classified according to ACMG guidelines. We detected C5 mutations in colorectal cancer (3 samples carried APC and TP53 mutations), 2 C5 and a C4 mutation in melanoma. Breast and prostate cancer samples showed respectively 12 and 9 C3 variants, while no C5 mutations were found. Deeper analysis is ongoing and more data will be available soon.

Conclusions: TSO500 confirmed its efficacy for the identification of somatic, germline variants, TMB and MSI determination in tumorigenic samples and can be relevant for an improved patient's management and stratification for target therapies.

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P12.061.A Development of a miRNA-based classifier for molecular colorectal cancer subtypes

Ronja S. Adam[1,2], *Dennis Poel*[3], *Leandro Ferreira Moreno*[1,2], *Joey Spronck*[1], *Tim R. de Back*[1,2], *Florian Markowetz*[4], *Xin Wang*[5,6], *Henk M. W. Verheul*[3], *Tineke E. Buffart*[7], *Louis Vermeulen*[1,2]

[1]Center for Experimental and Molecular Medicine (CEMM) and Cancer Center Amsterdam, Amsterdaum UMC, Amsterdam, Netherlands, [2]Oncode Institute, Amsterdam, Netherlands, [3]Department of Medical Oncology, Radboud University medical center, Nijmegen, Netherlands, [4]Cancer Research UK Cambridge Institute, University of

Cambridge, Cambridge, United Kingdom, [5]Department of Biomedical Sciences, City University of Hong Kong, Hong Kong, Hong Kong, [6]Shenzhen Research Institute, City University of Hong Kong, Shenzhen, China, [7]Netherlands Cancer Institute, Department of Gastrointestinal Oncology, Amsterdam, Netherlands.

Colorectal cancer (CRC) has been classified into Consensus Molecular Subtypes (CMSs) with implications for our understanding of tumour heterogeneity and the prognosis of patients. This classification is based on the expression of protein coding genes, messenger RNAs (mRNAs). MicroRNAs (miRNAs) have also been shown to play a role in tumour heterogeneity and CMS-specific biology. In contrast to mRNAs, they have a smaller size and increased stability, making miRNA expression quantification more easily accessible. Therefore, we built a miRNA-based CMS-classifier by translating the existing mRNA-based CMS-classifier using machine learning. The performance of this miRNA-assigned CMS-classifier (CMS-miRaCl) was validated in two independent data sets. To gain insight into the biological relevance, we evaluated its most important features. We found that miRNAs previously reported to be relevant in microsatellite instable CRCs or in Wnt-signalling were important features for CMS-miRaCl. With further validations proving its robustness, this miRNA-based alternative might allow to implement CMS-classification in clinical workflows more easily.

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P12.062.B Germline copy number variants: an underreported genetic diagnosis in gastrointestinal tumour risk syndrome suspected individuals

J. Garcia-Pelaez*[1,2,3], *S. Laurie**[4], *G. Demidov**[5], *I. Paramonov*[6], *A. K. Sommer*[7], *I. B. A. W. te Paske*[8], *D. Huntsman*[9], *ERN-GENTURIS group, SOLVE-RD consortium, E. Holinski-Feder*[10], *M. Tischkowitz*[11], *S. Aretz*[7,12], *N. Hoogerbrugge*[8], *R. M. de Voer*[8], *C. Oliveira*[1,2,3], ** contributed equally*

[1]Instituto de Investigação e Inovação em Saúde (i3S), Universidade do Porto, Porto, Portugal, [2]Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), Porto, Portugal, [3]Faculty of Medicine, University of Porto (FMUP), Porto, Portugal, [4]Centre Nacional d'Anàlisi Genòmica-Centre de Regulació Genòmica (CNAG-CRG), Parc Científic de Barcelona, Barcelona, Spain, [5]Institute of Medical Genetics and Applied Genomics, University of Tübingen, Tübingen, Germany, [6]Department of Clinical and Molecular Genetics and Rare Disease, Hospital Universitari Vall D'hebron, Barcelona, Spain, [7]Institute of Human Genetics, Medical Faculty, University of Bonn, Bonn, Germany, [8]Department of Human Genetics, Radboud university medical center, Radboud Institute for Molecular Life Sciences, Nijmegen, Netherlands, [9]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada, [10]MGZ - Medizinisch Genetisches Zentrum, Munich, Germany, [11]Academic Laboratory of Medical Genetics and National Institute for Health Research Cambridge Biomedical Research Centre, University of Cambridge, Cambridge, United Kingdom, [12]National Center for Hereditary Tumor Syndromes, University Hospital Bonn, Bonn, Germany.

Germline pathogenic variants, including rare copy number variants (CNVs) in cancer predisposing genes (CPG), cause genetic tumour risk syndromes (TRS). TRS-causative variants can be clinically actionable and lead to intensive surveillance and/or risk reducing surgery that improve morbidity and mortality. Regrettably, causative and actionable variants cannot be found for all TRS-suspected individuals. While for SNV-calling specificity/sensitivity is almost 100%, CNV detection in exome-data remains challenging. We hypothesized that pathogenic CNVs in CPG may solve some of the yet unexplained, but clinically suspected gastrointestinal TRS-cases. The ERN-GENTURIS/SOLVE-RD project, re-analyzed exomes from 293 unsolved TRS-cases: adenomatous polyposis (AP; n = 105), hyperplastic polyposis (HP; n = 98), hereditary gastric cancer (HGC; n = 83) and hereditary colorectal cancer (hCRC; n = 7). CNVs were called with four different variant callers (ClinCNV, ExomeDepth, Conifer, VarGenius). 341 CNVs filtered from 229 CPGs were prioritized for their involvement in GI tumours, quality and calling by >1 caller. High-quality and/or 'multiple-called' CNVs were evaluated using IGV and focused paired-end mapping/split-read analysis. Eight CNVs (6-del; 2-dup), 3 'multiple-called', were found in 11/293 TRS-cases, sometimes in cases with an atypical phenotype. CNVs found in *CDH1* (n = 1), *PALB2* (n = 1), *APC* (n = 2), *MSH6* (n = 1) are under validation with qPCR and MLPA. The *CDH1* deletion found in 4 HGC-relatives, supported by split-reads/paired-end mapping, was considered an actionable diagnosis (4.8% among HGC cases). A deletion affected *PALB2* in 1/83 (1.2%) HGC cases. Two deletions affected *APC* in 2/105 (2%) AP cases. Altogether, this approach delivered a potential diagnosis in 3.8% unsolved GI TRS-cases, that may become actionable after lab/clinical validation.

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P12.063.C Harnessing segment breakpoint statistics to interpret copy number variation in cancer

Qingyao Huang*, Michael Baudis*

University of Zurich, Zurich, Switzerland.

Genomic variations are direct cause of tumor formation and accomplice in its continuous evolution. While point mutations can be pinpointed to a targeted genetic element, copy number variations (CNVs) involve copy number gain or loss of a large DNA segment which often covers hundreds of genetic elements in one event. Although futile variations commonly exist in cancer and serve as an incubator for its subtype evolution, we observe consistency in CNV landscape within the same cancer types and corresponding increase in heterogeneity along with increased distinction in physiology and morphology. This implies that particular CNV may promote cancer type-specific progression. A focal CNV (of length shorter than 3Mb) has indicated stronger biological relevance. In addition, we observe highly frequent overlap of breakpoints in samples of the same cancer type. Taken together, we designed a statistic that harness information of CNV segment breakpoint, to delineate the importance of genetic elements covered by CNV segments. Using the TCGA ovarian, lung and breast adenocarcinoma data, we have identified their cancer type-specific oncogenes and tumor suppressor genes on the top of the ranking. The comparison with GISTIC2.0 using the same data showed similar regional peaks, but our method can provide significance measure on the finer gene level. With the confirmatory results on the known tumor promoting genes, this work has a potential to identify novel functional pathways that are exerted through CNV. We expect to expand this method to the non-coding area to provide a better overview of the CNV functional landscape.

Q. Huang: None. **M. Baudis:** None.

P12.064.D Expression Profiling of Jak-STAT Pathway Under Cutaneous T-Cell Lymphoma Early Stages UVB and PUVA Treatment

Irina V. Kozlova, *Dmitry A. Verbenko, Anastasiya A. Vorontsova, Arfenya E. Karamova, Marian B. Zhilova, Alexey A. Kubanov*

State Research Center for Dermatovenerology and Cosmetology of Russian Ministry of Health, Moscow, Russian Federation.

Introduction: Mycosis fungoides (MF) is the most common subtype of cutaneous T-cell lymphomas. Activation of the signal transducers and activators of transcription (STAT) protein family have been found to involved in the pathogenesis of leukemias, Hodgkin lymphomas and CTCL.

Materials and Methods: The expression levels of JAK-STAT pathway genes in affected vs nonaffected skin were studied, as well gene expression profiling at affected skin during UVB-311 and PUVA therapy of MF. F*OXP3, GATA3, IRF4* and *NFKB1* gene transcript levels were also evaluated. The study included 20 MF patients diagnosed using clinical examination and histopathological and immunohistochemical skin biopsies tests. RNA expression was evaluated in skin biopsies by qPCR using taqman probes with StepOne5 equipment.

Results: The *JAK3, STAT1* and *FOXP3* expression levels in affected vs visually normal skin were elevated in 75% patients, whereas lowered expression of *GATA3* shown for 90% of patients. The expression fold change heatmap revealed three clusters with distinct expression patterns in patients. PUVA treatment elevated transcription level of *GATA3, STAT5B*, and *JAK1* on 62-75% of patients; *JAK3, STAT1, STAT4* and *FOXP3* expression is decreased in 75-85% of patients. Almost the same shaping shown after UVB treatment, but *STAT4* elevated in 57% of patients.

Conclusions: JAK-STAT genes reveals different pattern of expression in affected vs non-affected skin of MF patients. The level of genes expression is changed during UV therapy towards the visually normal skin values; however the expression of *JAK1* and *JAK3* genes is arose for one third of patients, indicating possible disease stage advance.

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P12.065.A Exploring a mutation agnostic ctDNA-based workflow for early response monitoring in HR positive, HER2 negative Metastatic Breast Cancer

Alessandra Franzoni[1], *Lucia Bortot*[2], *Carla Corvaja*[2], *Lorenzo Allegri*[3], *Roberta Mazzeo*[2], *Lorenza Palmero*[2], *Debora Basile*[2], *Elisa Bertoli*[2], *Silvia Buriolla*[2], *Martina Alberti*[2], *Giada Targato*[2], *Elisa De Crignis*[2], *Lucia Da Ros*[4], *Stefania Russo*[5], *Paola Di Nardo*[4], *Marta Bonotto*[5], *Barbara Belletti*[4], *Gustavo Baldassarre*[4], *Lorenzo Gerratana*[2,4], *Fabio Puglisi*[2,4], *Giuseppe Damante*[1,2]

[1]*Institute of Human Genetics-ASU FC, Udine, Italy,* [2]*University of Udine, Udine, Italy,* [3]*Institute of Human Genetics, Udine, Italy,* [4]*IRCCS CRO National Cancer Institute, Aviano, Italy,* [5]*ASU FC, Udine, Italy.*

Background: Endocrine therapy (ET) is the mainstay treatment for HR positive, HER2 negative Metastatic Breast Cancer (Luminal MBC) but early disease dynamics is still an unmet need. The aim of the study was to proof the concept of a mutation-agnostic early disease monitoring based on circulating tumor DNA (ctDNA).

Methods: The study enrolled and characterized through ctDNA droplet digital PCR (ddPCR) 49 women with Luminal MBC. ctDNA% was defined as the proportion of the different lengths of ACTB DNA fragments (short/medium/long). Blood samples were collected before treatment start (BL) and after 3 months (E1). Matched pairs variations were tested through Wilcoxon test, the prognostic impact of ctDNA% and DNA yield was tested through Cox regression.

Results: The main treatment was ET+CDK4/6 inhibitors (92%). Pts with a ctDNA% > 75th percentile were burdened by a significantly worse outcome (Median PFS: 7.8 months, $P = 0.0290$). When compared with E1, a significant decrease in ACTB_short (71% $P = 0.0162$), ACTB_medium (66% $P = 0.0011$) and ACTB_long (78% $P = 0.0001$) was observed. The prognostic impact of a drop higher than 10% was then investigated for the different fragments of ACTB in terms of PFS. While a significant impact was observed for ACTB_short (HR: 5.98, 95%CI 1.61 - 22.24, $P = 0.008$), no significance was observed for ACTB_medium and ACTB_long. The prognostic impact of ACTB_short was also retained after multivariable analysis (HR: 4.88, 95%CI 1.07 -22.17, $P = 0.040$).

Conclusions: The study proofed the concept of a feasible mutation-agnostic workflow for a more granular disease monitoring and prognostication in MBC.

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P12.067.C DGCR8-E518K confirms a susceptibility allele for multinodular goiter in conjunction with peripheral schwannomatosis

Clara Nogué[1], *Èlia Grau*[2,3], *Joan Brunet*[2,4,5], *Anne-Sophie Chong*[6], *HyeRim Han*[1], *Eduard Dorca*[7], *Barbara Rivera*[1,6,8]

[1]*Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet de Llobregat, Barcelona, Spain,* [2]*Hereditary Cancer Program, Catalan Institute of Oncology, IDIBELL-IGTP-IDIBGI, L'Hospitalet de Llobregat, Badalona, Girona, Spain,* [3]*Program in Molecular Mechanisms and Experimental Therapy in Oncology (Oncobell), IDIBELL, Hospitalet de Llobregat, Spain,* [4]*Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), -, Spain,* [5]*Medical Sciences Department, School of Medicine, University of Girona, Girona, Spain,* [6]*Gerald Bronfman Department of Oncology, McGill University, Montreal, QC, Canada,* [7]*Bellvitge's Hospital, Department of Pathological Anatomy, L'Hospitalet de Llobregat, Barcelona, Spain,* [8]*Lady Davis Institute, The Jewish General Hospital, Montreal, QC, Canada.*

Introduction: Familial forms of multinodular goiter (MNG) are rare. Nowadays the only bona-fide susceptibility gene for fMNG is DICER1. Last year, we identified a single germline pathogenic variant in the nuclear microprocessor DGCR8 (c.1552G>A;p.E518K) that was responsible for a family featuring fMNG in conjunction with peripheral schwannomatosis (Rivera et al,. 2020). Later, a 35-year-old man with a history of multiple schwannomas ($n = 7$) since the age of 12, thyroid nodules, papillary thyroid cancer (dx.23y), and renal cyst (dx.33y) was being followed at the institute català d'oncologia. No family history of thyroid nodules or schwannomatosis was reported.

Methods: Blood from the affected proband and his non-affected sister and parents was collected jointly with patient's tissue of 5 schwannomas and 3 thyroid samples. Sanger-seq was performed in blood and tumor samples. Whole exome sequencing (WES) was performed in the germline DNA from the family members and in the DNA from the tumors.

Results: WES of the proband's blood DNA identified the c.1552G>A;p.E518K variant, whereas no other likely pathogenic variant was identified in any other cancer susceptibility genes. Sanger-seq showed loss of heterozygosity at the variant level in all analyzed tumors. Segregation analysis in the germline DNA of parents and sister revealed no other carriers and pointed to a de novo character of the variant. WES analysis of five schwannomas is currently ongoing.

Conclusions: The identification of this new case further confirms the role of DGCR8 as a novel tumor susceptibility gene adding papillary thyroid carcinoma to the syndrome associated phenotypes.

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P12.068.D Highly sensitive detection method of *DICER1* tumor hotspot mutations by drop-off droplet digital PCR

Roseline Vibert[1,2], *Marion Gauthier-Villars*[1,2], *Christelle Carrière*[1,2], *Catherine Dubois d'Enghien*[1,2], *Anne Vincent-Salomon*[2,3], *Dominique Stoppa-Lyonnet*[1,4], *Ivan Bièche*[1,4], *Emmanuelle Jeannot*[1,2,3], *Lisa Golmard*[1,2]

[1]*Service de Génétique, Institut Curie, Paris, France,* [2]*Université PSL, Paris, France,* [3]*Service d'Anatomo-Pathologie, Institut Curie, Paris, France,* [4]*Université de Paris, Paris, France.*

Introduction: DICER1 syndrome is an autosomal dominant inherited syndrome predisposing to various benign and malignant tumors, mainly occurring in children and young adults, requiring a broad surveillance starting at birth with repeated irradiating imaging exams and sedations for young patients. It is caused by germline pathogenic variants in the *DICER1* gene. More than 90% of tumors bear a *DICER1* missense hotspot mutation, as a second hit, involving one of six codons clustered in exons 24 and 25. We designed and validated in vitro a drop-off ddPCR system to scan all *DICER1* hotspot codons.

Materials and Methods: Three drop-off ddPCR assays were designed (one for exon 24, two for exon 25), with two TaqMan probes per assay, one complementary to the wild type sequence of the region containing hotspot mutations, another used as a reference. Eight tumor-derived DNAs and five synthetic oligonucleotides bearing *DICER1* hotspot mutations were tested.

Results: The 13 tested mutations were detected, with a limit of detection ranging from 0.07% to 0.31% for codons p.E1705, p.D1709 and p.D1713 in exon 24, and from 0.06% to 0.15% for codons p.G1809, p.D1810 and p.E1813 in exon 25.

Conclusions: The high sensitivity of this method is compatible with its use for plasma circulating tumor DNA (ctDNA) analysis for early tumor detection in DICER1 syndrome patients. It may reduce the need for radiation exposure and sedation in surveillance protocols. It may also improve patient prognosis. Clinical trials are needed to evaluate ctDNA analysis in DICER1 syndrome patients.
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P12.070.B DNA methylation status of circulating tumor DNA enables therapy response monitoring and assessment of tumor burden

Walter Pulverer[1], *Jagdeep Singh Bhangu*[2], *Silvia Schönthaler*[1], *Rudolf Öhler*[2], *Thomas Bachleitner-Hofmann*[2], *Andreas Weinhäusel*[1]

[1]*AIT Austrian Institute of Technology GmbH, Vienna, Austria,* [2]*Medical University of Vienna, Vienna, Austria.*

Introduction: Neoadjuvant chemotherapy (neoCTx) followed by hepatic resection is the treatment of choice of patients with colorectal cancer liver metastasis (CLM). Only about 70% of patients respond to neoCTx. Recent evidence suggests that cfDNA methylation is a highly sensitive biomarker and may more accurately reflect tumour burden and treatment response than conventional markers for CRC.

Methods: Thirty-four patients with CLM who received neoCTx prior to intended hepatic resection were included in this study. Peripheral blood plasma was collected at baseline and before each cycle of neoCTx. cfDNA was digested with 4 different methylation sensitive restriction enzymes and analysed for aberrant methylation of 48 CRC-associated genes. Methylation marker levels were correlated with baseline tumour volume and treatment response and compared with the standard tumour markers CEA and CA 19-9.

Results: The methylation markers SEPT9, DCC, BOLL and SFRP2 were present in all patients at baseline and displayed a stronger correlation with tumour volume than CEA and CA 19-9. Serial measurement of these methylation markers allowed for discrimination between operated and non-operated patients after 1 cycle of neoCTx. The early dynamic changes of SEPT9 and DCC also seemed to correlate with pathohistological response. Methylation values of eleven out of the 48 tested CRC-associated markers showed a strong correlation (>0.80) with SEPT9.

Conclusion: Our data suggest that serial measurements of CRC-associated methylation markers is a valuable tool for early response assessment. We also identified a set of eleven markers, who have the potential to strengthen the value of the CRC-marker SEPT9.

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P12.072.D Identification of the R882H mutation in DNMT3A by a restriction test in patients with hematologic neoplasms

Elizaveta Kulaeva[1], *Pavel Lipilkin*[2], *Tatyana Lipilkina*[3], *Elena Mashkina*[1]

[1]*Southern Federal University, Rostov-on-Don, Russian Federation,* [2]*Rostov State Medical University, Rostov-on-Don, Russian Federation,* [3]*Don State Technical University, Rostov-on-Don, Russian Federation.*

DNMT3A is one of the most frequently mutated genes in adult hematological neoplasms, including myelodysplastic syndrome (MDS), acute myeloid leukemia (AML) and chronic myeloid leukemia (CML). The most frequent somatic mutation in DNMT3A is R882H (c.2645G>A); this amino acid substitution reduces the enzymatic activity of the protein and destabilizes its functional tetrameric form in vitro and in vivo. Our work is focused on creating a method to identify the R882H somatic mutation in the DNMT3A gene for use in the diagnosis of MDS. We developed a specific restriction test using Fsp4HI restrictase and original primers and tested it on a sample of 81 patients with various hematological neoplasms: MDS (n =, AML (n = and CML (n =) to determine the frequency of the mutation in the MDS specifically. The sources indicated a frequency of 12,2% for R882H in MDS, frequency in all our sample was 2,47 ± 1,72%, but patients with this mutation had diagnosed CML, not MDS. We also identified a mutation in the second restriction site Fsp4HI, which was in the investigated DNA fragment at positions 113118-113122 (NG_029465.2) in three patients with AML, MDS, and CML accordingly. Therefore, we found that the R882H mutation is not

characteristic of MDS but is more likely characteristic of CML, which is important for clarifying the role of this mutation in the pathogenesis of myeloid neoplasms. We also found a new mutation that requires further study of its effect on enzyme function and the pathogenesis of MDS, AML and CML.

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P12.073.A Transcriptional profile of *NOMO1* deleted cells, a common alteration in early-onset colorectal cancer

Abel Jesús Martel-Martel[1,2], *Paula García-Vallés*[1,2], *Jésica Pérez-García*[1,2,3], *Luis Ángel Corchete*[1], *Nerea Gestoso-Uzal*[1,2], *Diego Iglesias-Corral*[1,2], *Juan Luis García*[1,3], *Ana Belén Herrero*[1,2,3], *José Perea*[4], *Rogelio González-Sarmiento*[1,2,3]

[1]*Biomedical Research Institute of Salamanca (IBSAL), Salamanca, Spain,* [2]*Molecular Medicine Unit, Department of Medicine, University of Salamanca, Salamanca, Spain,* [3]*Institute of Molecular and Cellular Biology of Cancer (IBMCC), University of Salamanca-CSIC, Salamanca, Spain,* [4]*Health Research Institute, Fundación Jiménez Díaz University Hospital, Madrid, Spain.*

Introduction: Early-onset colorectal cancer (EOCRC) incidence has been increasing in the last few years. About 10% of all colorectal cancer (CRC) tumors are diagnosed in this cohort of patients (under 45 years). In a recent study performed by our group, we have detected a homozygous deletion of the *NOMO1* gene in more than 70% of EOCRC patients, suggesting that *NOMO1* could be a molecular marker associated with EOCRC. Our aim was to identify, using whole genome microarray, differential gene expression between *NOMO1* Knockout and *NOMO1* wild type cell lines that could explain its role in colorectal carcinogenesis.

Material and methods: CRISPR/Cas9 technology was used to generate *NOMO1*-KO HT29 (CRC), HCT116 (CRC) and HS-5 (mesenchymal) cell lines. Total RNA from two *NOMO1*-KO and *NOMO1*-WT clones was labeled and hybridized according to Affymetrix protocols. Samples were hybridized to Clarium-S human array and scanned using GeneChip System of Affymetrix. WebGestalt tool was used for microarray data analysis.

Results: A total of 2 overexpressed genes (FC>1,5; *HSP90B1* and *PCNT*) and 8 infra-expressed genes (FC<-1,5; *NOMO1, PLPP3, RFT1, STEAP1B, FBXO32, SLC6A8, ZNF503* and *ATP8B1*) showed up in common between the three cell lines. Notably, PLPP3 is associated with the activation of the Wnt/β-catenin pathway, which plays an important role in the development of CRC.

Conclusions: The inactivation of NOMO1 leads to the differential expression of some genes that could explain its implication in the development of CRC. Validation of these mRNA expression changes and new functional experiments are being performed. Study funded by PI20/01569.

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P12.074.B Simultaneous detection of *EGFR* mutation and PD-L1 overexpression in a case of advanced lung adenocarcinoma

POMPILIA APOSTOL*, Alex Sebastian Pinzariu*

SC CLINICA SANTE SRL, BUCHAREST, Romania.

Worldwide, lung cancer represents the leading cause of cancer-related mortality, in spite of personalized therapy success. Testing for *EGFR/ALK* status and PD-L1 expression will help for guidance of treatment strategy. Simultaneous testing of *EGFR/ALK* driver mutations and PD-L1 expression, in order to gain valuable time for the treatment decision of advanced adenocarcinoma (ADC). Our study has included 26 unrelated Caucasians patients (20-84 years-old) with NSCLC (23 ADC and 3 squamous cell carcinoma). Genomic DNA was isolated from primary or secondary FFPE tumors samples, with 20-80% tumor cells content. *EGFR* status was analyzed by Real-Time PCR using a commercial kit, while *ALK* status and PD-L1 expression by IHC (clone D5F3, respectively SP 263). All investigated samples were ALK negative. By tumor proportion level (TPS), PD-L1 expression was classified as negative (TPS < 1%, n = 10), weak (TPS 1-49%, n = 11) and strong (TPS ≥50%, n = 5). Five patients presented driver mutations in exon 19 or 21 (3 ex19del and 2 Leu858Arg). It has to be mentioned that along with EGFR-Ex19del a patient has also a high PD-L1 overexpression (>80%). This is a 65-year-old female, which underwent last year a right upper lobectomy being diagnosed with micropapillary and acinary adenocarcinoma. Though the studied lot comprises only 26 patients, it can be seen that simultaneous testing of these biomarkers has beneficial effects both on the patient's turnaround time and on amount of biological material used. However, the most important benefit is the rapidity of treatment strategy guidance, a very important aspect for advanced/inoperable ADC patients.

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P12.075.C High sensitivity detection of endometrial cancer-associated genetic variants in minimally-invasive gynecological samples

Fátima Marín*[1], *Beatriz Pelegrina**[2], *Sònia Paytubi**[2], *Ferran Briansó*[3,4], *Paula Peremiquel*[2], *Jon Frias*[2], *Yolanda Benavente*[2], *José Manuel Martínez*[5], *Marc Barahona*[5], *Sergi Fernández*[5], *Alba Zanca*[6], *Nuria Baixeras*[6], *August Vidal*[6], *Axel Rodríguez*[6], *Júlia Canet*[1], *Álvaro Carmona*[2], *Javier de Francisco*[7], *Victor Caño*[7], *Francesc Xavier Bosch*[2], *Silvia de Sanjosé*[8], *Jordi Ponce*[5], *Gabriel Capellá*[1], *Xavier Matias-Guiu*[6], *Joan Brunet*[1,9], *Laia Alemany*[2], *Marta Pineda**[1], *Laura Costas**[2]

[1]*Hereditary Cancer Program, Catalan Institute of Oncology, Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), ONCOBELL Program, CIBERONC, L'Hospitalet de LLobregat, Spain,* [2]*Cancer Epidemiology Research Programme, Catalan Institute of Oncology, Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), CIBERESP, L'Hospitalet de LLobregat, Spain,* [3]*Department of Genetics, Microbiology and Statistics, Universitat de Barcelona, Barcelona, Spain,* [4]*Roche Diagnostics, Sant Cugat del Vallès, Spain,* [5]*Department of Gynecology, Hospital Universitari de Bellvitge, Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), L'Hospitalet de LLobregat, Spain,* [6]*Pathology, Hospital Universitari de Bellvitge, Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), L'Hospitalet de LLobregat, Spain,* [7]*Department of Anesthesiology. Hospital Universitari de Bellvitge, Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), L'Hospitalet de LLobregat, Spain,* [8]*National Cancer Institute, Bethesda, MD, USA,* [9]*Hereditary Cancer Program, Catalan Institute of Oncology, Institut d'Investigació Biomèdica de Girona (IDIBGI), Girona, Spain.*

Diagnosis of endometrial cancer (EC) is based on the histopathological assessment of endometrial biopsies among symptomatic women. Genetic analyses of minimally invasive samples using highly sensitive techniques offer a promising perspective for screening and early detection of EC. Our aim was to identify somatic mutations associated to EC that could be detected in cervical Pap Brush and cervico-vaginal self-samples. Sixty-one

DNA samples from nine EC cases and four from controls were extracted from endometrial biopsies, clinician-collected cervical samples, cervico-vaginal self-samples, and blood; plus surgical specimens for cases. A custom panel targeting exons of 49 genes, which incorporates duplex unique molecular identifiers, was used to sequence samples at a high depth. The deduplication process was performed using a combination of Picard, fgbio and bwa tools. VarDictJava and Mutect2, in tumor-only mode, were used for variant calling. Variants detected by both callers and with variant allele frequency (VAF)>0.5% were considered and filtered by quality and functional impact. A minimum VAF of 5% was set in aspirates to filter out low frequency variants of normal tissue. Genetic variants at VAF>5% in tumors (67 variants across the 9 tumors) were detected in paired endometrial aspirates, clinician-collected cervical samples, and vaginal self-samples with a sensitivity of 96% (60/67), 76% (53/67), 69% (49/67), respectively. No somatic variants were identified in gynecological samples from controls or blood from any women. This approach is useful to detect tumor somatic mutations in gynecological minimally-invasive samples at high sensitivity. A larger sample size is required to validate these results.

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P12.076.D Somatic testing in unsolved familial adenomatous polyposis cases increases diagnostic yield and discloses a high prevalence of *APC* mosaicism

Paula Rofes[1,2], *Jesús del Valle*[1,2], *Sara González*[1,2], *Lídia Feliubadaló*[1,2], *Marta Pineda*[1,2], *Eva Tornero*[1,2], *Mireia Menéndez*[1,2], *Xavier Muñoz*[1,2], *Eva Montes*[1], *Raquel Cuesta*[1], *Matilde Navarro*[1], *Ares Solanes*[1], *Núria Dueñas*[1], *Sílvia Iglesias*[1], *Mónica Salinas*[1], *Judith Balmaña*[3], *Joan Brunet*[1,2], *Gabriel Capellá*[1,2], *Conxi Lázaro*[1,2]

[1]*Hereditary Cancer, Catalan Institute of Oncology, ONCOBELL program, IDIBELL, L'Hospitalet de Llobregat, Spain,* [2]*Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Madrid, Spain,* [3]*High Risk and Cancer Prevention Unit, Medical Oncology Department, University Hospital Vall d'Hebron, Universitat Autònoma de Barcelona and Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain.*

Introduction: Familial adenomatous polyposis (FAP) is a highly penetrant dominant condition accounting for 1% of CRC cases. It is characterized by the development of hundreds to thousands of colorectal adenomas. Up to 90% of FAP cases are caused by inactivating mutations in *APC*, and mosaicism has been previously reported in 20% of sporadic cases. **Aim:** To explore the prevalence of mosaicism in 11 unexplained cases of FAP and to evaluate the impact of somatic testing on the diagnostic yield.

Methods: Paired samples of colorectal polyps, tumors and/or mucosa were analyzed using a custom NGS hereditary-cancer panel. Whenever possible, the extension of mosaicism within the different embryonic layers was examined.

Results: 14 polyposis-associated genes were analyzed in 26 colorectal samples by high-coverage sequencing. A mosaic pathogenic variant was identified in 7/11 patients (63.6%), in all cases in *APC*. In 2/7 (28.57%) mosaicism was restricted to colonic tissues, while in 5/7 (71.43%) it was extended to the blood. Mosaicism affected the germline in one patient, being his daughter a heterozygous carrier of the same variant.

Conclusions: The implementation of somatic testing in FAP patients identifies *APC* mutations previously missed, improving the diagnostic yield in our cohort of patients, attributable

to the identification of low-frequency germline variants as well as mosaic variants confined to the colon. **Grant support:** Carlos III Institute funded by FEDER–a way to build Europe– [PI19/00553; PI16/00563 and CIBERONC]; Government of Catalonia [2017SGR1282 and 2017SGR496].

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P12.077.A Development of *APC*-specific ACMG/AMP variant classification guidelines

Isabel Spier[1,2], *Xiaoyu Sherry Yin*[3,4], *John-Paul Plazzer*[3], *Andreas Laner*[5], *Ian M. Frayling*[6], *Deborah Ritter*[7,8], *Sharon Plon*[7,8], *Marc Greenblatt*[9], *Johan T. den Dunnen*[10], *Finlay A. Macrae*[3,11], *Stefan Aretz*[1,2], *on behalf of the InSiGHT - ClinGen Hereditary Colon Cancer / Polyposis Variant Curation Expert Panel*

[1]*Institute of Human Genetics, Medical Faculty, University of Bonn, Bonn, Germany,* [2]*National Center for Hereditary Tumor Syndromes, University Hospital Bonn, Bonn, Germany,* [3]*Department of Colorectal Medicine and Genetics, Royal Melbourne Hospital, Parkville, Australia,* [4]*Melbourne Medical School, University of Melbourne, Parkville, Australia,* [5]*Department of Genomics, Medical Genetics Center Munich, Munich, Germany,* [6]*Institute of Medical Genetics, University Hospital of Wales, Cardiff, United Kingdom,* [7]*Baylor College of Medicine, Houston, TX, USA,* [8]*Texas Children's Cancer Center, Baylor College of Medicine, Houston, TX, USA,* [9]*Vermont Cancer Center, University of Vermont College of Medicine, Burlington, VT, USA,* [10]*Department of Human Genetics and Clinical Genetics, Leiden University, Leiden, Netherlands,* [11]*Department of Medicine, University of Melbourne, Parkville, Australia.*

Introduction: The proper characterisation of the clinical significance of germline variants is of high relevance to reduce the amount of variants with uncertain clinical significance (VUS). Pathogenic *APC* variants are causative for Familial adenomatous polyposis, a colorectal cancer predisposition syndrome. This project aims to improve variant classification by the development of *APC*-specific classification criteria within the approval process of a Hereditary Colon Cancer / Polyposis Variant Curation Expert Panel (VCEP) from the International Society for Gastrointestinal Hereditary Tumours (InSiGHT) and ClinGen.

Methods: *APC*-specific adaptations of the interpretation guidelines published by the American College of Medical Genetics and the Association of Molecular Pathology (ACMG/AMP) were suggested based on expert opinions, database analyses, and literature search conducted by the VCEP.

Results: Four of the 28 original criteria were left unchanged, whilst six were not used for different reasons. For the remaining 18 criteria, gene- or disease-based specifications and/or evidence strength modifications were made. The main changes concern the "pathogenic very strong" (PVS1) criterion (specifications at the 5′ and 3′ end of the gene), and modifications of the minor allele frequency thresholds (criteria BA1, BS1 and PM2). The validation of

the *APC*-specific modifications by systematic evaluation of known variants is currently underway.

Conclusions: It is expected that using the *APC*-specific guidelines will improve variant interpretation and facilitates resolution of variants with conflicted assertions in ClinVar. Based on the adapted allele frequency thresholds, it is likely that in particular a considerable portion of VUS can be reclassified as (likely) benign.

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P12.078.B Variant classification and expert curation: *APC* as a pilot project and model of the collaborative InSiGHT-ClinGen Hereditary Colon Cancer / Polyposis (ICCP) Variant Curation Expert Panel (VCEP)

Stefan Aretz[1,2], *Isabel Spier*[1,2], *Xiaoyu Sherry Yin*[3], *John-Paul Plazzer*[3], *Elke Holinski-Feder*[4,5], *Johan T. den Dunnen*[6], *Deborah Ritter*[7,8], *Sharon Plon*[7,8], *Marc Greenblatt*[9], *Ian Frayling*[10], *Finlay A. Macrae*[3,11], *on behalf of the InSiGHT - ClinGen Hereditary Colon Cancer / Polyposis Variant Curation Expert Panel*

[1]*Institute of Human Genetics, Medical Faculty, University of Bonn, Bonn, Germany,* [2]*National Center for Hereditary Tumor Syndromes, University Hospital Bonn, Bonn, Germany,* [3]*Department of Colorectal Medicine and Genetics, Royal Melbourne Hospital, Parkville, Australia,* [4]*MGZ - Medical Genetics Center, Munich, Germany,* [5]*Arbeitsgruppe erbliche gastrointestinale Tumore, Medizinische Klinik und Poliklinik IV – Campus Innenstadt, Klinikum der Universität München, Munich, Germany,* [6]*Department of Human Genetics and Clinical Genetics, Leiden University, Leiden, Netherlands,* [7]*Baylor College of Medicine, Houston, TX, USA,* [8]*Texas Children's Cancer Center, Baylor College of Medicine, Houston, TX, USA,* [9]*Vermont Cancer Center, University of Vermont College of Medicine, Burlington, VT, USA,* [10]*Institute of Medical Genetics, University Hospital of Wales, Cardiff, United Kingdom,* [11]*Department of Medicine, University of Melbourne, Parkville, Australia.*

Characterising the clinical significance of germline variants becomes imminent for the translation of genetic testing into medical practice. The realisation of this goal is a complex process that includes curation of locus-specific databases (LSDB), the implementation of Variant Curation Expert Panels (VCEP), and the development of standardized rules for variant classification such as gene-specific modifications of the ACMG/AMP guidelines. We describe this process for the curation and classification of *APC* variants which is part of a long-term endeavour of the International Society for Gastrointestinal Hereditary Tumours (InSiGHT) in collaboration with the Clinical Genome Resource (ClinGen). To improve data centralisation and standardisation, we identified *APC*-related LSDBs with thousands of rare or private variants and initiated the removal or merging of outdated or orphan ones. A novel version of the reference InSiGHT *APC* LSDB was established. Subsequently, variants were reclassified and annotation inconsistencies within and between the *APC* and ClinVar databases were scrutinised and partly solved. As a collaborative effort, an InSiGHT-ClinGen Hereditary Colon Cancer/Polyposis VCEP is constituted in the ClinGen Hereditary Cancer Clinical Domain. This includes the development and validation of *APC*-specific adaptations of the generic ACMG/AMP interpretation guidelines (separate abstract), which will improve in particular the classification of the high amount of sequence variants with unknown clinical significance (VUS) in public uncurated resources such as ClinVar. Prioritised VUS will be classified by the VCEP regularly, whose consensus will represent the most authoritative classification of pathogenicity. The InSiGHT-ClinGen effort might serve as a model for other variant interpretation initiatives.

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P12.079.C Frequency of pathogenic variants in Fanconi anemia genes in hereditary breast and ovarian cancer families

Vida Stegel[1], *Gašper Klančar*[1], *Nina Anžič*[1], *Petra Škerl*[1], *Vita Šetrajčič Dragoš*[1], *Alenka Bombač*[1], *Anja Zagožen Klasinc*[1], *Vesna Vogrič*[1], *Mateja Krajc*[2], *Ana Blatnik*[2], *Ksenja Strojnik*[2], *Marta Banjac*[2], *Srdjan Novaković*[1]

[1]*Department of Molecular Diagnostics, Institute of Oncology Ljubljana, Ljubljana, Slovenia,* [2]*Cancer Genetics Clinic, Institute of Oncology Ljubljana, Ljubljana, Slovenia.*

Introduction: It is estimated that 5-10% of breast and ovarian cancers (BC/OC) have hereditary origin. Higher BC/OC risk is mostly attributed to pathogenic variants (PV) in *BRCA1/2* genes, as well as to PV in other high to moderate BC/OC risk genes as listed by NCCN guidelines for hereditary breast and ovarian cancer syndrome HBOC (NCCN-HBOC genes). Several of NCCN-HBOC genes belong to Fanconi anemia (FA) pathway. There are reports on PVs in other FA genes (nonNCCN-HBOC FA genes) found in cancer patients, but their association with cancer risk is not yet fully established. The aim of this study was to analyze a cohort of HBOC families to determine the frequencies of PVs in nonNCCN-HBOC FA genes in NCCN-HBOC PV positive and NCCN-HBOC PV negative HBOC cases.

Methods: Since 2015 at our institute, the individuals fulfilling the HBOC testing criteria were counseled and screened for germline PVs in NCCN-HBOC genes by Illumina's NGS multigene panel. Retrospective reanalysis of nonNCCN-HBOC FA genes (*FANCA*, *FANCB*, *FANCC*, *FANCD2*, *FANCE*, *FANCF*, *FANCG*, *FANCI*, *FANCL*, *FANCM*, *SLX4*, *ERCC4*) was performed on 2023 individuals from different HBOC families. Statistical analysis was performed using Fisher's exact test.

Results: In NCCN-HBOC genes 466 PVs and in nonNCCN-HBOC FA genes 37 PVs were identified. Of all nonNCCN-HBOC FA genes *FANCM*, *FANCD2* and *FANCA* were most frequently mutated.

Conclusions: The percentage of subjects with PVs in nonNCCN-HBOC FA genes is statistically significantly higher among the NCCN-HBOC PV negative individuals compared to the NCCN-HBOC PV positive individuals (2.16 vs 0.65%, $p = 0{,}0294$).

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P12.080.D Familial Colorectal Cancer Type X syndrome: new insights

Felipe A. O. Garcia[1], *Edilene S. de Andrade*[1], *Henrique C. R. Galvão*[2], *Cristina S. Sabato*[3], *Natalia Campacci*[1], *Matias E. Melendez*[4], *Rui M. Reis*[1], ***Edenir I. Palmero***[4,1]

[1]*Molecular Oncology Research Center, Barretos Cancer Hospital, Barretos, Brazil,* [2]*Oncogenetics Department, Barretos Cancer*

Hospital, Barretos, Brazil, [3]Center of Molecular Diagnosis, Barretos Cancer Hospital, Barretos, Brazil, [4]Pele Little Prince Research Institute, Curitiba, Brazil.

Abstract: Familial colorectal cancer type X (FCCTX) is a heterogeneous colorectal cancer predisposition syndrome that differs from Lynch syndrome in that it does not present microsatellite instability and exhibits the four main genes of mismatch repair, MLH1, MSH2, MSH6, and PMS2 function. Besides, its genetic etiology remains to be elucidated. We performed whole-exome sequencing of constitutive material from 38 cancer-affected patients from 33 families at risk for FCCTX (following Amsterdam I clinical criteria). The variant classification followed the American College of Medical Genetics and Genomics (ACMG) guidelines. A total of seven pathogenic/likely pathogenic variants in six unrelated families (18.2% of the families) were identified. Most of the genes were involved in DNA damage repair. One of them is the known CRC predisposing gene *CHEK2*. In addition, pathogenic/likely pathogenic variants were found in genes previously associated with FCCTX/hereditary CRC as *OGG1* and *FAN1*. Furthermore, potentially pathogenic variants were identified in the *TREX1, ASXL1, PRKN* and *SLX4* genes. Although the association of these variants needs to be further studied, we were able to present new potential genes that might be involved in the carcinogenesis of FCCTX, thus providing important clues that can contribute to the understanding of hereditary colorectal cancer genetic basis.

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P12.081.A Interactome of FGFR1 mutations found in low-grade gliomas (LGGs)

HyeRim Han[1], *Cassandra Jin-Wong*[2], *Tenzin Gayden*[3], *Geoffrey Hesketh*[2], *Michael Slattery*[4], *Nada Jabado*[3], *William D. Foulkes*[5,6], *Anne-Claude Gingras*[2], *Barbara Rivera*[1,6]

[1]Bellvitge Institute for Biomedical Research (IDIBELL), L'Hospitalet de Llobregat, Spain, [2]Department of Molecular Genetics, Gingras Lab, University of Toronto, Toronto, ON, Canada, [3]Department of Pediatrics, The Research Institute of the McGill University Health Center, Montreal, QC, Canada, [4]Department of Microbiology and Immunology, McGill University, Montreal, QC, Canada, [5]Cancer Research Program, Research Institute, McGill University Health Centre, Montreal, QC, Canada, [6]Gerald Bronfman Department of Oncology, McGill University, Montreal, QC, Canada.

Introduction: Aberrant FGFR signaling is involved in the development of many different types of pathologies, including those of the brain. In particular, oncogenic hotspot FGFR1 mutations p.N546K and p.K656E have been demonstrated to play a critical role in the etiology of low-grade gliomas (WHO Grade I/II).

Material and methods: We performed a comprehensive genotype-phenotype correlation of 739 cases of different FGFR1-mutated pathologies published in public databases and literature. Furthermore, we investigated the MAPK/ERK pathway activation of FGFR1 single and double mutants found in low-grade brain tumors, and identified potential interactors through co-immunoprecipitation assays and Bio-ID.

Results: Targeted analysis of CNS-related pathologies in the comprehensive genotype-phenotype correlation revealed that a majority of the brain tumors (200/290, 69%) concentrate on p.N546K and p.K656E mutations. While appearing predominantly as single mutants, FGFR1 double mutants appear significantly higher in combination with p.K656E (31/39, 79%, $p = 0.0001$). Molecular biology experiments indicate that while both the single and double FGFR1 mutants activate the MAPK/ERK pathway, double mutants modulate the signaling levels. Furthermore, BioID results demonstrate that the germline p.R661P mutant retains similar interactors as that of wild-type FGFR1, while the double mutants share many with p.N546K, recapitulating the interactome of this activating mutant.

Conclusions: Genotype-phenotype correlation demonstrates the prevalence of p.N546K and p.K656E mutations in brain tumors, in particular with p.K656E *in cis* for double mutants. Interrogation of the proximity interactome of FGFR1 mutants revealed potential treatment targets against low-grade gliomas with impaired FGFR signaling components.

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P12.082.B Analyzing clinical behavior of two germline variant in FH gene in a young female patient with renal cancer hereditary syndrome (HLRCC)

Jesica M. Ramirez, *Elizabeth Fader Kaiser, Hernán Rodríguez Zanini, Miriam Rogel*

Mendoza Central Hospital, Mendoza, Argentina.

Hereditary leiomyomatosis and renal cell carcinoma (HLRCC) is an autosomal dominant hereditary cancer syndrome. It is characterized by the development of uterine and cutaneous leiomyomas, renal cell carcinoma (RCC) and rarely uterine leiomyosarcomas. The RCC occurrence is low and it affects young adults whom generally present metastases at diagnosis. This tumor is usually unilateral, aggressive and the main histological subtype being type 2 papillary RCC. HLRCC is due to mutations of the fumarate hydratase (*FH*, fumarase) gene that encodes for FH enzyme which acts as a tumor suppressor. At date about 100 mutations have been reported in a few families. The aim of this work is to report the presence of two variants, in a germline testing, in a 34 years female patient who consulted with diagnosis of RCC in advanced stage and cutaneous leiomyomas. Her mother and sister presented cutaneous leiomyomas confirmed by biopsy. The FH gene was analyzed by exome sequencing (NGS) using Illumina platform. Genetic testing reported two variants in exon 8 of FH gene. First variant, c.1157A>C, p.Gl386Pro was classified as probably pathogenic according functional assays. This mutation is very rare in the general population and was predicted by in silico software to be deleterious. The second variant, c.1157A>G, p.Gl386Arg was classified as probably pathogenic according to LOV.3 and CLINVAR. Considering to low prevalence, no clear correlation between mutations (genotype) and phenotype has been found. This report could contribute to increase de knowledge. Cosegregation studies will be considering to certificate variable expressivity and penetrance in relatives.

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P12.083.C The transcription factors TFEB and TFE3 promote tumor growth in Birt-Hogg-Dube' syndrome

CHIARA DI MALTA[1,2], *Angela Zampelli*[1], *Letizia Granieri*[3], *Luisa Lanfrancone*[3], *Andrea Ballabio*[1,2,4]

[1]*Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli (NA), Italy,* [2]*Medical Genetics Unit, Department of Medical and Translational Science, Federico II University, Naples, Italy,* [3]*European Institute of Oncology (IEO), Milan, Italy,* [4]*Department of Molecular and Human Genetics, Baylor College of Medicine., Houston, TX, USA.*

The Birt-Hogg-Dube' (BHD) syndrome is an autosomal dominant inherited disorder due to loss of function germline mutations in the folliculin (FLCN) gene. Symptoms include benign cutaneous fibrofolliculoma, pulmonary and kidney cysts and spontaneous pneumothoraces. The most severe manifestation of the disease are kidney tumors, which develop in about one out of three affected individuals due to a somatic second-hit mutation leading to the inactivation of the remaining FLCN allele. However how FLCN suppresses tumor establishment has remained elusive for decades. The MiT/TFE family members TFEB and TFE3 are constitutively nuclear in cells depleted of FLCN and we recently demonstrated that genetic depletion of TFEB completely rescued renal cyst formation, restored normal kidney function and lifespan of kidney-specific FLCN KO mice, thus pointing at TFEB as a key driver of renal pathology in BHD. However, the contribution of TFEB, or the one of its paralogue TFE3, to the development of kidney cancer associated with this disorder has never been addressed. We now show that depletion of TFEB, or the one of TFE3, remarkably reduced the growth of a BHD-patient-derived tumor cell line in vivo. Moreover, gene expression studies allowed us to identify key TFEB/TFE3 downstream pathways which could be responsible for tumorigenesis in BHD. Our findings demonstrate the relevance of constitutive activation of TFEB and TFE3 in the growth of kidney tumors associated with BHD syndrome and encourage future studies exploiting TFEB/TFE3 inhibitors as a therapy for these tumors.

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P12.084.D Searching for novel fusion genes by RNA-Seq in follicular lymphoma

Maria Pospelova[1], *Konstantin Danilov*[1], *Ekaterina Bozhokina*[2,3], *Yuriy Krivolapov*[2], *Anna Gorbunova*[2], ***Igor Evsyukov***[1]

[1]*ITMO University, Saint-Petersburg, Russian Federation,* [2]*Mechnikov North-West State Medical University, Saint-Petersburg, Russian Federation,* [3]*Institute of Cytology RAS, Saint Petersburg, Russian Federation.*

Follicular lymphoma is the second most common non-Hodgkin's lymphoma. In most cases, tumor cells contain a t(14;18) translocation that activates *BCL2*, a key gene for apoptosis. However, there are a number of minor genetic alterations. Identifying such events in a particular patient can affect the diagnosis, prognosis and treatment of cancer. Therefore, the search for new fusion genes is very important for a personalized approach in medicine.

Currently, the search for new fusion genes can be performed in RNA-Seq data using special programs, such as STAR-Fusion. Found by this approach and subsequently validated hybrid genes can be used for diagnostic purposes using FISH, Southern blotting and RT-PCR.

In this study whole transcriptome RNA-sequencing profiling was performed on biopsy specimens obtained from patients with untreated follicular lymphoma and non-tumor lymph nodes. Altogether only 3 fusion genes were found in 18 control samples, while in 19 tumor samples 32 fusion events were detected. Among identified fusion genes we found known follicular lymphomas fusions such as *BCL2/IGH* and *BCL6/IGH* with high level of evidence. There are also fusions typical for other cancer types, e.g. *KDM5A/NINJ2* (breast adenocarcinoma). Most of the fusions are not covered in databases (ChimerKB, FusionGDB), but participating genes are well known in carcinogenesis. Found fusion genes will be validated by Sanger sequencing.

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P12.087.C Identification of new genes involved in germline predisposition to early-onset gastric cancer

Cristina Herrera-Pariente[1], *Roser Capó-García*[1], *Marcos Díaz-Gay*[1,2], *Sabela Carballal*[1], *Jenifer Muñoz*[1], *Joan Llach*[1], *Ariadna Sánchez*[1], *Laia Bonjoch*[1], *Coral Arnau-Collell*[1], *Yasmin Soares de Lima*[1], *Mariano Golubicki*[3,4], *Gerhard Jung*[1], *Juan José Lozano*[5], *Antoni Castells*[1], *Francesc Balaguer*[1], *Luis Bujanda*[6], *Sergi Castellví-Bel*[1], *Leticia Moreira*[1]

[1]*Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD), Gastroenterology Department, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain,* [2]*Department of Cellular and Molecular Medicine, University of California, San Diego, CA, USA,* [3]*Oncology Section, Hospital of Gastroenterology "Dr. C. B. Udaondo", Buenos Aires, Argentina,* [4]*Molecular Biology Laboratory, Hospital of Gastroenterology "Dr. C. B. Udaondo", Buenos Aires, Argentina,* [5]*Bioinformatics Platform, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas yDigestivas (CIBEREHD), University of Barcelona, Barcelona, Spain,* [6]*Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD), Gastroenterology Department, Biodonostia Health Research Institute, Basque Country University (UPV/EHU), San Sebastián, Spain.*

Introduction: The genetic cause for several families with gastric cancer (GC) aggregation is unclear, with marked relevance in early-onset patients. We aimed to identify new candidate genes involved in GC germline predisposition.

Material and Methods: Whole-exome sequencing (WES) of germline samples was performed in 20 early-onset GC patients without previous germline mutation identified. WES was also performed in nine tumor samples to analyze the somatic profile. Sequencing germline data were filtered to select variants with plausible pathogenicity, rare frequency and previously involved in cancer. Then, a manual filtering was performed to prioritize genes according to current knowledge and function. These genetic variants were prevalidated with Integrative Genomics Viewer (IGV). A selection step was carried out and Sanger sequencing was performed.

Results: 274 genetic variants located on 205 genes were prioritized. After IGV and selection step, 58 genetic variants in 52 different candidate genes were validated by Sanger sequencing (Table 1).

Conclusion: Among them, *APC*, *FAT4*, *CTNND1* and *TLR2* seem to be the most promising genes because of their role in hereditary cancer syndromes, tumor suppression, cell adhesion and *Helicobacter pylori* recognition, respectively. These results will be helpful to increase the knowledge of predisposition to early-onset gastric cancer. Grant Support: CIBEREHD Beca de investigador novel 2017-2019, Fondo de Investigación Sanitaria/FEDER (17/00878, 20/

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Table 1. Final candidate genes to gastric cancer predisposition.

Function	Genes
Germline cancer predisposition	*APC*, *ATM*, *SDHC*, *COL7A1*, *GPC3*, *RNF43*, *EXT2*, *POLD1*, *EXT1*, *POT1*, *PTCH1*, *POLH*, *GATA2*, *BAP1*, *ERCC2* and *FANCA*
Cell adhesion	*FAT1*, *FAT2*, *FAT4* and *CTNND1*
Tumor suppression genes	*PHF2*, *LARP7*, *LRP1B*, *WWOX*, *ADAMTS9*, *LATS1*, *BCL6B*, *ITIH5*, *NEO1*, *MAD1L1*, *GPX7*, *IQGAP2*, *DACT2*, *SIRT3*, *RCC1* and *EPHB2*
Helicobacter pylori recognition	*IL12A*, *TLR1*, *TLR2*, *TLR5*, *TLR10*, *A4GNT* and *MUC1*
DNA repair	*UNG*, *KAT5*, *RAD23A*, *HBP1* and *LIG3*
Other related functions	*ARID4A*, *ARID1B*, *ATP4A* and *ROBO1*

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P12.088.D Somatic mutations of epigenetic regulation genes in gastric cancer

Alexey Kalinkin[1], *Ekaterina Kuznetsova*[2], *Ekaterina Alekseeva*[1], *Tatyana Deryagina*[1], *Irina Bure*[2], *Dmitry Mikhaylenko*[2], *Alexander Tanas*[1], *Vladimir Strelnikov*[1], *Marina Nemtsova*[2]

[1]*Research Centre for Medical Genetics, Moscow, Russian Federation,* [2]*I.M. Sechenov First Moscow State Medical University, Moscow, Russian Federation.*

Background: Epigenetic processes play a significant role in carcinogenesis, cancer recurrence and metastasis, and may serve as useful clinical biomarkers. Analysis of epigenetic regulator genes mutation landscape in gastric cancer (GC) samples may reveal novel clinical biomarkers and therapeutic targets.

Methods: We investigated somatic mutations of 25 epigenetic regulation genes, using the NGS panel in 95 GC samples. All identified somatic variants wereverified in tumor and non-tumor tissue by Sanger sequencing. Prediction ofsomatic variants pathogenicity and impact on structure were carried out using PolyPhen2, SIFT, PROVEAN, MutPred2, I-Mutant 3 and HOPE3D tools. Westudied the survival of patients using the Kaplan-Meier method.

Results: For further analysis, we selected frameshift, nonsense or missensevariants with MAF < 0.0005 and not annotated in the ClinVar or dbSNP databases. Based on these criteria, we identified 60 somatic mutations in 45/95(48%) GC samples. 23/60 (38%) of the identified variants are new, not described in the databases. 28/60 mutations we identified as pathogenic, 9/28(32%) were verified in non-neoplastic tissue, and were not described germline variants. We showed that survival was significantly reduced in patients with T3-4 tumor size to have mutations, compared with the T3-4 group without mutations ($p = 0.043$) and patients with distant metastases (M1) to have mutations, compared with M1 group without mutations ($p < 0.0001$). It was also revealed that mutations in ARID1A prevail in patients with distant metastases ($p = 0.03$). Although clinical associations are somewhat premature, since the group of patients is not very large yet. This work was supported by the Russian Foundation for Basic Research (project No.18-29-09020).

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P12.089.A Functional characterisation of *PLK2* polymorphism

Pia Pužar Dominkuš, *Petra Hudler*

University of Ljubljana, Faculty of Medicine, Institute of Biochemistry and Molecular Genetics, Ljubljana, Slovenia.

Introduction: The majority of gastric cancer (GC) tumours is characterized by chromosomal instability and extreme molecular heterogeneity, which can be driven by low-penetrating changes, including single nucleotide variants (SNVs) in cell cycle genes. The aim of our study was to functionally investigate SNVs in candidate genes *CDC20*, *PLK2*, *PLK3* and *ATM*.

Methods: 542 GC patients and healthy controls were included in the genotyping study. Survival analysis was performed using the Kaplan-Meier method. The binding of candidate miRNAs on polymorphic allele was evaluated with Luciferase reporter assay.

Results: Analyses of genetic models revealed significant associations with GC risk: male patients with *PLK2*-rs963615 CT genotype had lower risk, whereas female patients had higher risk (OR = 0.59, 95%CI = 0.37-0.93, $P = 0.023$; OR = 2.03, 95%CI = 1.09-3.80, $P = 0.026$, respectively). *PLK3*-rs12404160 AA genotype was associated with higher risk in male population (OR = 3.55, 95% CI = 1.26-10.04, $P = 0.015$). Patients with the *CDC20*-rs710251 CC had shorter overall survival (53.7 months) in comparison to patients with AC or AA genotypes (131.7 and 133.5 months). For patients with the *PLK2*-rs15009 GG genotype the 10-year survival rate was 63.5% in comparison to patients with CG or CC genotypes (15.7% and 20%). Relative luciferase activity of the pmirGLO-PLK2-3'UTR-Var (G allele) was decreased for 35% when cells were co-transfected with miR-23b-5p mimic in comparison to the pmirGLO-PLK2-3'UTR-Wt (C allele).

Conclusion: The *CDC20*-rs710251 and *PLK2*-rs15009 could potentially be useful for survival prediction. MiR-23b-5p directly targets *PLK2*-rs15009 G allele. Supported by YR and P1-390 grants from the Slovenian Research Agency (ARRS).

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P12.090.B Solving the genetic aetiology of hereditary gastrointestinal cancer - a collaborative multicentre endeavour within the project Solve-RD

Anna K. Sommer[1], *Iris B. A. W. te Paske*[2], *José García-Peláez*[3,4,5], *Andreas Laner*[6], *Elke Holinski-Feder*[6,7], *Verena Steinke-Lange*[6,7], *Sophia Peters*[1], *Laura Valle*[8], *Isabel Spier*[1,9], *Solve-RD consortium, Carla Oliveira*[3,4,5], *Richarda M. de Voer*[2], *Nicoline Hoogerbrugge*[*2], *Stefan Aretz*[*1,9], * *contributed equally*

[1]*Institute of Human Genetics, Medical Faculty, University of Bonn, Bonn, Germany,* [2]*Department of Human Genetics, Radboud university medical center, Radboud Institute for Molecular Life Sciences, Nijmegen, Netherlands,* [3]*Instituto de Investigação e Inovação em Saúde (i3S), Universidade do Porto, Porto, Portugal,* [4]*Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), Porto, Portugal,* [5]*Faculty of Medicine, University of Porto (FMUP), Porto, Portugal,* [6]*MGZ - Medizinisch Genetisches*

Zentrum, Munich, Germany Arbeitsgruppe erbliche gastrointestinale Tumore, Medizinische Klinik und Poliklinik, Munich, Germany, [7]Campus Innenstadt, Klinikum der Universität München, Munich, Germany, [8]Hereditary Cancer Program, Catalan Institute of Oncology, IDIBELL and CIBERONC, Barcelona, Spain, [9]National Center for Hereditary Tumor Syndromes, University Hospital Bonn, Bonn, Germany.

Background: Patients and families with suspected, but genetically unexplained (unsolved) genetic tumour risk syndromes lack appropriate treatment and prevention, leading to preventable morbidity and mortality. To tackle this problem, patients from the European Reference Network on Genetic Tumour Risk Syndromes (ERN GENTURIS) are analysed in the European Commission´s research project "Solving the unsolved rare diseases" (Solve-RD). The aim is to uncover known and novel cancer predisposing genes by reanalysing available whole-exome sequencing (WES) data of large cohorts in a combined manner, and applying a multidimensional omics approach.

Approach: Nearly 500 genetically unsolved cases with suspected hereditary gastrointestinal tumour syndromes (polyposis, early-onset/familial colorectal cancer and gastric cancer) from multiple European centres were included. Currently, clinical and germline WES data from 294 cases are analysed. Furthermore, an extensive molecular profiling of gastrointestinal tumours is planned and deep learning techniques will be applied. The ambitious, multidisciplinary project is accompanied by a number of methodical, technical, and logistic challenges, which require the development and implementation of new analysis tools, standardisation of bioinformatics pipelines, and strategies to exchange data and knowledge.

Results and Outlook: The re-analysis of 229 known cancer predisposition genes allowed solving 2-3% of GENTURIS cases. Further variants in 7% of the cases are under revision. The integration of expert knowledge and new technologies will help to identify the genetic basis of a substantial number of additional cases within the ongoing project. The ERN GENTURIS approach might serve as a model for other genomic initiatives. Solve-RD received funding from EU Horizon 2020 (No.779257).

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P12.091.C Genetic susceptibility to prostate cancer biochemical recurrence after radical prostatectomy

Anca Pavel[1,2], *Danai Stambouli*[1], *Adrian Preda*[3], *Ismail Gener*[3], *Gabriela Anton*[2]

[1]*Cytogenomic Medical Laboratory, Bucharest, Romania,* [2]*Stefan S. Nicolau Institute of Virology, The Romanian Academy, Bucharest, Romania,* [3]*Center of Internal Medicine-Nephrology, Fundeni Clinical Institute, Bucharest, Romania.*

Introduction: Radical prostatectomy is an effective treatment for localized prostate carcinoma. Although the majority experience long term disease control, a significant percentage of patients will develop biochemical recurrence after surgery. The aim of this study was to determine if biochemical recurrent disease can be related to a particular risk genotype. Therefore, a single genetic variant was selected in order to determine if genotyping could provide information regarding the outcome of prostate cancer.

Materials and Methods: A total of 50 Romanian men diagnosed with prostate cancer that underwent radical prostatectomy were included in the study. DNA was extracted from peripheral blood from all 50 patients. We divided the prostate cancer patients in two groups according to biochemical recurrence (biochemical recurrent disease and no biochemical recurrence). Genotyping of the rs4054823 C/T polymorphism, located on the chromosome 17p12, was performed by allelic discrimination with Taqman 5'-nuclease assays.

Results: Statistical analysis revealed a significant correlation of TT genotype with an increased risk for biochemical recurrence. Kaplan-Meier survival curves of recurrence-free survival after radical prostatectomy showed a significant difference between the TT genotype compared to CC/CT genotypes ($P = 0.0362$).

Conclusions: Our findings propose the TT genotype as a potential susceptibility factor for biochemical recurrence of prostate cancer after radical prostatectomy. Therefore, detection of hereditary genetic variations associated with prostate cancer progression and outcome has a significant impact on the accurate classification of the disease, providing insight into possible preventive and therapeutic targets.

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P12.092.D High frequency of clinically actionable pathogenic variants detected with an On-Demand 35-gene panel in Hereditary Breast and Ovarian Cancer Syndrome in Castilla y León (Spain)

Mar Infante[1], *Enrique Lastra*[2], *Luis E. Abella*[3], *Noemy Martínez*[1], *Lara Hernández*[1], *Mercedes Durán*[1]

[1]*Cancer Genetics Group. Instituto de Biología y Genética Molecular (UVa-CSIC), VALLADOLID, Spain,* [2]*Unit of Genetic Counseling in Cancer. Complejo Hospitalario de Burgos, Burgos, Spain,* [3]*Unit of Genetic Counseling in Cancer. Hospital Universitario Rio Hortega, VALLADOLID, Spain.*

Introduction: BRCA1 and BRCA2 are the high-risk genes that are traditionally screened for germline mutations in the context of Hereditary Breast and Ovarian Cancer Syndrome (HBOC). With Next Generation Sequencing, detection of mutations in other predisposing genes using multigene panels is rapid and cost-effective. Thus, we have designed an On-Demand gene panel, including 35 genes associated with HBOC to improve our clinical diagnostic routine, especially in those families that gathers several cancer types, which do not fit into a specific inherited cancer syndrome.

Patients and Methods: 48 HBOC cancer Patients were screened for mutations using the On-Demand Research Assay. Library and template preparations were performed using the automated Ion Chef System, then sequenced in Ion S5 with Ion 520 Chip according to the manufacturer's instructions.

Results: After variant filtering, 37 variants were described: 12 Pathogenic or Likely Pathogenic variants were identified in 6 different genes (5 in *ATM*, 2 in *BRCA2*, 2 in *TP53* and one for *BRCA1*, *FANCM* and *PALB2*, each). All but one, are actionable genes according with Clinical guidelines. 25 Unclassified Variants were identified. Two variants in *ATM* and *FANCM* are novel.

Conclusions: Our 35- genes Panel allowed us to detect clinically actionable variants in a third of the families studied. They would have gone overlooked if we only have screened BRCAs genes. Moreover, the workflow used increased the number of UV, but in an affordable way to further research.

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P12.093.A Molecular characterization of two new cell lines derived from head and neck squamous cell carcinomas

*Nerea Gestoso-Uzal[1,2,3], Juan Luis García[2,3], Javier Fernández-Mateos[1,2], **Sonia Carretero-Domínguez**[1,2,3], María Ovejero-Sánchez[1,2,3], Pedro Blanco Pérez[2,4], Luis Angel Corchete[2,3], Ana Belén Herrero[1,2,3], Juan Jesús Cruz-Hernández[1,2,5], Rogelio González-Sarmiento[1,2,3]*

[1]Molecular Medicine Unit, Department of Medicine, University of Salamanca, Salamanca, Spain, [2]Biomedical Research Institute of Salamanca (IBSAL), University Hospital of Salamanca-USAL-CSIC, Salamanca, Spain, [3]Institute of Molecular and Cellular Biology of Cancer (IBMCC), University of Salamanca-CSIC, Salamanca, Spain, [4]Department of Otorhinolaryngology, University Hospital of Salamanca-IBSAL, Salamanca, Spain, [5]Medical Oncology Service, University Hospital of Salamanca-IBSAL, Salamanca, Spain.

Background: Head and neck squamous cell carcinoma (HNSCC) includes epithelial malignancies of the oral cavity, pharynx and larynx. Most of the HNSCC cell lines reported do not come from the primary tumour site and its molecular characterization is not available. The aim of this work was to characterize new cell lines that can be used as models to study HNSCC from different locations.

Patients and methods: Two cell lines were newly established from oropharyngeal (32816) and laryngeal (32860) squamous cell carcinomas. Their characterization was done by karyotyping, FISH analysis, array-based comparative genomic hybridization and microarray expression profiling.

Results: Karyotyping of the cells lines by G banding revealed a moderate hyperploidy for both cell lines. Also, we identified an unbalanced translocation between chromosomes 3 and 12 in 32816, confirmed by FISH. Loss of heterozygosity and copy number variations were detected. Both cell lines share certain alterations associated with HNSCC, but we also found small regions specifically altered for each line. The study of the transcriptome showed 1313 genes with differential expression between 32816 and 32860. According to p-value we selected the most significantly dysregulated genes as candidates for study: *NEDD4L*, *KLK6*, *GALNT14* and *SPARC*.

Conclusions: We established two new cell lines derived from different HNSCC locations that are good models to study this type of cancer. The characterization of the lines showed some common alterations already described in HNSCC, as well as differences that can be due to the location of the primary tumours.

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P12.094.B Characterization of recurrent breakpoints in head and neck cancer

Nerea Gestoso-Uzal[1,2,3], *Juan Luis García[2,3], Javier Fernández-Mateos[1,2], Luis Angel Corchete[2,3], Sonia Carretero-Domínguez[1,2,3], Paula García-Vallés[1,2,3], Abel Martel-Martel[1,2], Edel Del Barco[2,4], Ana Belen Herrero[1,2,3], Rogelio González-Sarmiento[1,2,3], Juan Jesús Cruz-Hernández[1,2,4]*

[1]Molecular Medicine Unit, Department of Medicine, University of Salamanca, Salamanca, Spain, [2]Biomedical Research Institute of Salamanca (IBSAL), University Hospital of Salamanca-USAL-CSIC, Salamanca, Spain, [3]Institute of Molecular and Cellular Biology of Cancer (IBMCC), University of Salamanca-CSIC, Salamanca, Spain, [4]Medical Oncology Service, University Hospital of Salamanca-IBSAL, Salamanca, Spain.

Background: Genome-wide somatic DNA copy number aberration (CNA) profiling is a widely established approach to characterize chromosomal aberrations in cancer genomes. Evidence is emerging that structural chromosomal aberrations represent a biologically and clinically relevant class of alterations in many cancer types including solid tumours. The aim of this work was to identify the CNA-associated breakpoints in a large series of head and neck squamous cell carcinomas (HNSCC) and correlate them with clinical data. **Patients and methods**: Tumour samples from 177 patients included in the clinical trial TTCC-2007-01 were used. Tumours were analysed by OncoScan® assay and the computational method "GeneBreak" that identifies chromosomal breakpoint locations using DNA copy number profiles. Statistical analysis was performed to associate the genes showing recurrent breakpoints with clinical and molecular data, considering statistically significant $p < 0,05$.

Results: We detected 10688 breakpoints associated with genes. We selected 14 genes that were recurrently affected by breaks (FDR < 0.1) and showed break points in at least 25% of cases. Breakpoint in *SHANK2* gene was associated with *TP53* mutations. Tumours located in hypopharynx and larynx were linked with break in *CSMD1* gene. Histologically non-keratinizing tumours were associated with breaks in *RGS7* and *EMCN* genes. Number of focal changes was associated with *SHANK2* break. Overall survival (OS) was inversely correlated with the break in *LRP1B* gene.

Conclusion: We conclude that CNA-associated chromosomal breaks within genes represent a highly prevalent and clinically relevant subset of somatic variants (SVs) in HNSCC.

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P12.095.C Combinatorial effect of sorafenib, valproic acid and metformin in a hepatic cancer cells model

Edgar X. Franco-Juárez[1], *Alain Jesús Aguirre-Vázquez[1], Vianey Gonzalez-Villasana[2], Mario Bermúdez-de León[1]*

[1]Instituto Mexicano del Seguro Social, Monterrey, Mexico, [2]Universidad Autonoma de Nuevo León, Monterrey, Mexico.

Introduction: Hepatocellular carcinoma is the main type of hepatic cancer, which has the sixth and fourth place in incidence and mortality worldwide, respectively. Chemotherapeutic scheme of treatment extends the overall survival in three months. Moreover, latest research with drugs used in diseases other than cancer, like the histone deacetylases inhibitors or rapalogues, have been described with in vitro anticancer properties. These drugs have shown synergistic effects towards apoptosis induction and inhibition of angiogenesis, migration and proliferation in combination with the approved chemotherapeutic drug, sorafenib. In this study we evaluated the viability, migration and angiogenic potential using different combinations of sorafenib, valproic acid (VPA) and metformina in hepatic cancer model.

Materials and Methods: HepG2 cells were treated with different concentrations and combinations of sorafenib, VPA and

metformin for 48 h to evaluate viability with alamar blue assay, migration with a wound healing assays, and angiogenic potential by RT-qPCR assays with Taqman probes spanning on *VEGF-A* and *GAPDH* genes.

Results: Treatment with sorafenib, VPA or metformin shown viability reduction in a dose-dependent fashion and the combination of 2 μM sorafenib, 4 mM VPA and 10 mM metformin showed strongest synergistic effect in the viability reduction. This concentration also reduced migration but not *VEGF-A* gene expression compared to control.

Conclusion: Combination of sorafenib, VPA and metformin has synergistic effects towards reduction of viability and migration potential, in contrast with angiogenic potential, where levels of *VEGF-A* transcript remain with no changes.

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P12.096.D Multigene panel testing for hereditary breast and ovarian cancer syndrome: experience from a tertiary referral hospital

Miriam Potrony[1,2], *Blai Morales-Romero*[1], *Lorena Moreno*[3], *Belen Pastor*[4], *Jose L. Villanueva-Cañas*[5], *Celia Badenas*[1,2], *Isabel Aragón Manrique*[6], *Pilar Carrasco Salas*[7], *Paula Aguilera*[8,2], *Laura Ferrer-Mileo*[4], *Lydia Gaba*[4], *Barbara Adamo*[4], *Josep Oriola*[1], *Aurora Sánchez*[1], *Joan A. Puig-Butillé*[5,2]

[1]*Biochemical and Molecular Genetics Department, Hospital Clínic de Barcelona, IDIBAPS, Barcelona, Spain,* [2]*Centro de Investigación Biomédica en Red en Enfermedades Raras, Instituto de Salud Carlos III, Barcelona, Spain,* [3]*Gastroenterology Department, Hospital Clínic de Barcelona, Barcelona, Spain,* [4]*Medical Oncology Department, Hospital Clínic de Barcelona, Barcelona, Spain,* [5]*Molecular Biology CORE, Hospital Clínic de Barcelona, Barcelona, Spain,* [6]*Genetic Counseling Unit, Medical Oncology Department, Hospital Juan Ramón Jiménez, Huelva, Spain,* [7]*Genetic Unit, Clinical Analysis Department, Hospital Juan Ramón Jiménez, Huelva, Spain,* [8]*Dermatology Department, Hospital Clínic de Barcelona, IDIBAPS, Barcelona, Spain.*

Introduction: The implementation of next-generation sequencing in the clinical setting has increased the diagnostic yield of Hereditary Breast and Ovarian Cancer Syndrome (HBOC). We aimed to assess the spectrum of germline pathogenic variants in cancer susceptibility genes among Spanish patients selected for personal and/or family history of breast, ovarian, prostate, melanoma, and other *BRCA*-associated cancers.

Materials and Methods: The study cohort comprised 1381 Spanish patients referred to Hospital Clinic of Barcelona for genetic testing. The HBOC genes *BRCA1, BRCA2, PALB2, ATM, CHEK2, BRIP1, RAD51C, RAD51D, TP53*, and *PTEN*, and the Lynch Syndrome genes *MLH1*, *MSH2*, and *MSH6* were analyzed by commercial *Hereditary Cancer* Panels (Illumina) and an in-house bioinformatics pipeline.

Results: We detected that 9% and 3% of patients carried pathogenic variants in high-risk and moderate-risk genes, respectively. Three patients carried pathogenic variants in Lynch Syndrome genes and four patients carried pathogenic variants in two genes. Pathogenic variants prevalence was 12% in breast/ovarian cancer patients, 15% in prostate cancer, 10% in melanoma, and 6.6% in healthy individuals with a family history of *BRCA*-associated cancers. Individuals with two different HBOC tumors ($OR = 6.29$, $p < 0.001$) or family history of ovarian cancer ($OR = 2.08$, $p = 0.009$) were more likely to carry deleterious variants in high-risk genes.

Conclusions: In our clinical setting, the diagnostic yield of HBOC by multigene panel testing was 12%. This multigene testing strategy allows the identification of individuals carrying multiple pathogenic variants and the diagnosis of multiple hereditary cancer syndromes, resulting in a direct benefit for the families.

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P12.097.A Unravelling genetic predisposition to familial breast and ovarian cancer: identification of new susceptibility genes by case-control study

Alejandro Moles-Fernández[1], *Ester Aguado-Flor*[1], *Cristina Zamarreño*[1], *María Antolín*[2], *Sandra Bonache*[1], *Adrià López-Fernández*[1], *Lídia Feliubadaló*[3,4], *José Fernández-Navarro*[1], *Conxi Lázaro*[3,4], *Judith Balmaña*[1], *Orland Díez*[1,2], *Sara Gutiérrez-Enríquez*[1]

[1]*Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain,* [2]*Vall d'Hebron University Hospital, Barcelona, Spain,* [3]*Catalan Institute of Oncology (ICO), Institut d'Investigació Biomédica de Bellvitge (IDIBELL), Hospitalet de Llobregat, Barcelona, Spain,* [4]*Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Madrid, Spain.*

Multiple high- and moderate-risk genes for breast and ovarian cancer have been identified. However, a high percentage of affected patients and families still remain genetically undiagnosed. Although different candidate genes have been identified, the evidence for association with the disease is still insufficient and the missing heritability remains unexplained. Our goal was to identify and validate candidate genes for hereditary breast and ovarian cancer (HBOC) using a case-control study approach. The exomes of 25 patients from 13 high-risk HBOC families as well as 63 candidate genes of 192 HBOC patients without pathogenic variants identified were sequenced. After variant annotation, we selected genes in which deleterious variants were identified according to their function and the segregation of the variant in affected relatives. *RRBP8*, *TPMT*, *MACROD1*, *DMC1*, *RALGDS*, and *TDP2* were identified as candidate genes for analysis in a case-control study. *EDC4* and *RECQL5* genes were included from collaborators' publications. DNA samples were collected from 500 healthy women and 1500 patients with HBOC without detected pathogenic variants in risk genes. To date, after massively amplicon sequencing, annotation and interpretation, a higher frequency of deleterious variants is observed in *RECQL5* in 250 patients compared to 50 controls and gnomAd database. These first results suggest *RECQL5* as a potential risk gene associated with HBOC, and the need to increase case-controls cohorts to verify this association. Funding: Carlos III Institute funded by FEDER-a way to build Europe- [PI16/01218-PI19/01303;PI19/00553;PI16/00563 and CIBERONC]; AECC; ERAPERMED2019-215 support and Government of Catalonia [2017SGR1282 and 2017SGR496].

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P12.098.B Massively parallel functional analysis of missense variants in the breast/ovarian cancer gene *RAD51C*

Gemma Montalban, *Larissa Milano, Amélie Rodrigue, Yan Coulombe, Sylvie Desjardins, Martine Dumont, Charles Joly-Beauparlant, Penny Soucy, Jean-Yves Masson, Jacques Simard*

CHU de Québec - Université Laval, Québec, QC, Canada.

Introduction: A notable proportion of hereditary breast/ovarian cancer cases are due to inherited pathogenic mutations in DNA repair genes. The application of multi-gene panels for genetic testing has led to an increased detection of variants of unknown clinical significance (VUS). Our work aims to implement a massively parallel functional approach to study the molecular impact of all possible missense substitutions in the *RAD51C* gene.

Materials and Methods: A *RAD51C* mutagenesis library was designed and cloned into an inducible, recombinase-site containing vector. In parallel, HeLa "landing pad" cells were generated, ensuring the recombination of one variant per cell. A subset of the library was integrated and cells were treated with PARP inhibitors. Genomic DNA from pre-treated and post-treated samples was collected and sequenced in a MiSeq instrument.

Results: To date, ~160 *RAD51C* missense variants have been screened. All variants were detected in the pre-treated pool at a similar abundance, confirming their optimal integration and expression. Variant read counts were reduced for the positive controls after treatment, confirming the synthetic lethal effect of olaparib when *RAD51C* is not functional. Experimental replicates and calculation of loss-of-function scores using other DNA damaging agents is ongoing.

Conclusions: We have developed a large-scale functional approach to measure the impact of all missense variants in the *RAD51C* gene using PARP inhibitors sensitivity as a readout. Future work will focus on validating our data with published works, clinical databases and complementary assays. The final goal is to improve the interpretation of *RAD51C* VUS and accelerate their clinical translation.

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P12.099.C Hereditary Cancer Predisposition Testing in Luxembourg

Karin Segers[1,2], *Marizela Kulisic*[1], *Ben Flies*[1], *Christian Müller*[1], *Philippe Theis*[1], *Karin Dahan*[1], *Barbara Klink*[1], *Daniel Stieber*[1]

[1]*National Center of Genetics (NCG), Laboratoire National de Santé (LNS), Dudelange, Luxembourg,* [2]*Human Genetics Department CHU Sart Tilman, Liège, Belgium.*

At our center, we provide oncogenetic consultations and molecular genetic testing for hereditary tumor diseases for whole Luxembourg. Here we report on the outcome of one year hereditary cancer predisposition testing using the Hereditary Cancer Solution developed by Sofia Genetics, an NGS-based capture panel optimized for the detection of SNVs, InDels, and CNVs in 26 genes. In 2020, we analyzed blood samples of altogether 276 patients. We identified pathogenic germline variants in 32 of 276 samples (positivity rate of 11.6%). Of those, 74.5% were identified in patients with a referral for suspicion of Hereditary Breast and Ovarian Cancer syndrome (HBOC). Fifty percent of pathogenic variants affected the BRCA1 and BRCA2 genes. The majority of non-BRCA variants were found in ATM and in MMR genes. The following cancer types had the highest positivity rates: triple negative breast cancer (23.3%), ovarian cancer (26.5%), and prostate cancer (40%). As expected, patients with triple negative breast cancer and ovarian cancer had pathogenic variants in BRCA1, BRCA2, ATM and RAD51. Interestingly, we identified a pathogenic germline variant in four of eleven patients with a personal history of prostate cancer. The affected genes -- PALB2, ATM, MSH2 and PMS2 -- are not known to be high prostate cancer risk genes. Distribution of affected genes and the positivity rate of 11.6% that we found in our cohort are in line with observations from the literature.

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P12.100.D Variant and gene prioritization strategy to identify new hereditary colorectal cancer genes by NGS

Mariona Terradas, *Isabel Quintana, Noemí González-Abuín, Pilar Mur, Matilde Navarro, Joan Brunet, Gabriel Capellá, Laura Valle*

Hereditary Cancer Program, Catalan Institute of Oncology; Oncobell Program, IDIBELL, Hospitalet de Llobregat, Spain.

Introduction: In the past two decades, multiple studies have been undertaken to elucidate the genetic cause of the predisposition to mismatch repair (MMR)-proficient nonpolyposis colorectal cancer (CRC); first by genome-wide linkage analysis and, nowadays, using next-generation sequencing techniques. Nevertheless, a relevant proportion of familial CRC cases remain unexplained. Here we propose a fast and focused variant selection strategy to facilitate the identification of MMR-proficient nonpolyposis CRC predisposing genes.

Material and methods: Peripheral blood DNA from 25 individuals belonging to 16 families affected with MMR-proficient nonpolyposis CRC was used to perform WES. After variant calling with GATK, rare (population MAF < 0.1%), predicted pathogenic variants in genes involved in pathways or processes relevant in colorectal carcinogenesis and hereditary cancer, including DNA Repair, Wnt and TGF-beta pathways (n = 2957 genes), were selected. Gene prioritization was then performed considering data from CanVar (data from 1000 familial/early-onset CRC patients), IntOGen (information on cancer driver genes), GTEx (gene expression), OMIM and Pubmed.

Results: Based on the filtering and variant prioritization strategies mentioned above, 24 candidate CRC-predisposing genes were selected. Validation in further >1000 unrelated familial/early-onset and sporadic CRC patients is currently being performed and final results will be presented at the meeting.

Conclusions: The variant prioritization strategy followed facilitated the identification of variants in relevant genes, being particularly useful in cases where only one (or two) affected member(s) is (are) sequenced; thus showing a higher performance than processes applied in the past to similar studies.

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P12.101.A Hereditary leiomyomatosis and renal cell carcinoma:identification and characterization of a new Spanish founder mutation in the *FH* gene

Estela Dámaso[1], *Ana Beatriz Sánchez Heras*[1], *Yoel Esteve*[1], *Virginia Díez Obrero*[2], *María-Isabel Castillejo*[1], *Adela Castillejo*[1], *Jose-Luis Soto*[1]

[1]*Hospital General Universitario Elche, Elche, Spain,* [2]*Institut d`Investigació Biomèdica de Bellvitge (IDIBELL), L'Hospitalet de Llobregat, Barcelona, Spain.*

Hereditary-leiomyomatosis-renal-cell-carcinoma (HLRCC) syndrome is a rare autosomal dominant disease caused by germline

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mutations in the fumarate hydratase (FH) gene. A highly recurrent HLRCC-associated missense variant [*FH:* c.1118A>G; p.(Asn373Ser)] was observed in apparently unrelated families from the southeast of Spain. Our goal was to establish the founder effect of this alteration and characterized its associated clinical phenotype.

Materials-Methods: Haplotype construction was performed using 37 flanking *FH* polymorphic markers covering ≈14 Mb. Twenty unrelated carriers were selected for genotyping. Clinical data from a recently published large Spanish cohort of HLRCC (PMID_33167498) was used to assess the clinical phenotype of this founder mutation and to look for genotype-phenotype associations, including 104 individuals with this mutation and 93 individuals carrying other *FH* pathogenic variants.

Results: Haplotype analysis confirmed that families shared a common haplotype (25/37 markers) between 0.61-0.82 Mb (≈1.40-1.89 cM). These results strongly suggest that recurrent *FH:* c.1118A>G variant observed in apparently unrelated individuals was indeed inherited from a founder ancestor. Patients carrying the founder mutation were diagnosed of cutaneous leiomyomas (CLM) 65%, uterine leiomyomas (ULM) 98%, renal cysts (RCy) 42% and renal cancer (RC) 10%. In addition, we found higher frequencies of CLMs, ULMs and RCys, than those with loss-of-function variants ($p < 0.005$).

Conclusions: Identification and characterization of founder mutations provide accurate and specific information regarding their penetrance and expressivity. Individuals carrying *FH:* c.1118A>G variant had lower frequency of RC than previously published in HLRCC, and higher frequencies of CLMs, ULMs and RCys when compared to HLRCC-individuals with loss-of-function variants.

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P12.102.B Gene panel tumor testing in ovarian cancer patients significantly increases the yield of clinically actionable germline variants beyond *BRCA1/BRCA2*

Ana Barbosa[1], *Pedro Pinto*[1], *Ana Peixoto*[1], *Joana Guerra*[1], *Carla Pinto*[1], *Catarina Santos*[1], *Manuela Pinheiro*[1], *Carla Escudeiro*[1], *Carla Bartosch*[1], *João Silva*[1], *Manuel R. Teixeira*[1,2]

[1]*Portuguese Oncology Institute of Porto (IPO-Porto), Porto, Portugal,* [2]*Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, Portugal, Porto, Portugal.*

Introduction: Since the approval of PARP inhibitors for the treatment of high-grade serous ovarian cancer that *BRCA1* and *BRCA2* genetic testing have therapeutic implications (germline and somatic variants) in addition to cancer risk assessment and should be offered to these patients at diagnosis irrespective of family history. However, variants in other genes besides *BRCA1* and *BRCA2* are associated with ovarian cancer predisposition, which would be missed by a genetic testing aimed only at indication for PARP inhibitor treatment. In this study, we aimed to evaluate the yield of clinically actionable germline variants using next-generation sequencing customized panel.

Material and methods: Next generation sequencing was performed in formalin-fixed paraffin-embedded tumor samples obtained from 96 patients diagnosed with ovarian cancer using a customized panel containing ten genes (*BRCA1*, *BRCA2*, *BRIP1*, *MLH1*, *MSH2*, *MSH6*, *PMS2, RAD51C*, *RAD51D* and *TP53*).

Results: In addition to 13.7% of deleterious germline *BRCA1/BRCA2* carriers, we identified 7.4% additional patients with pathogenic germline variants in other genes predisposing for ovarian cancer, namely *RAD51C*, *RAD51D* and *MSH6*, representing 35% of all pathogenic germline variants.

Conclusions: The strategy of reflex gene-panel tumor testing enables the identification of clinically actionable germline variants in a significantly higher proportion of ovarian cancer patients, which may be a valuable information in patients with advanced disease that have run out of approved therapeutic options. Furthermore, this approach increases the chance to make available genetic counseling, presymptomatic genetic testing, and gynecological cancer prophylaxis to female relatives who turn out to be healthy carriers of deleterious germline variants.

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P12.103.C Genetic causes of sarcomas development in young patients

Nathalia de Angelis de Carvalho*, Karina Miranda Santiago, Joyce Maria Lisboa Maia, Maria Nirvana da Cruz Formiga, Diogo Cordeiro de Queiroz Soares, Daniele Paixão Pereira, Felipe D'Almeida Costa, Dirce Maria Carraro, Giovana Tardin Torrezan*

AC Camargo Cancer Center, Sao Paulo, Brazil.

Introduction: Although genetic screening of cancer predisposing genes (CPGs) is currently well established for the most common hereditary tumors, there are a number of rare tumors, including sarcomas, which may be associated with hereditary cancer syndromes but whose pathogenic variants frequencies in these genes are still unknown. Aims: Define the frequency of pathogenic rare germline variants in known CPGs in young patients (<40 years) with sarcomas; evaluate the molecular characteristics of these tumors and search for new associated genes.

Methods: We evaluated 156 young patients diagnosed with sarcoma for the presence of germline variants using a custom 113 gene panel and Next-Generation Sequencing (NGS). Analyzes of protein expression and evaluation of loss of heterozygosity (LOH) in tumor tissue are being performed in cases with pathogenic variants.

Results: Pathogenic/likely pathogenic (P/LP) variants were detected in 31/156 patients (19.8%). These P/LP variants were found in genes previously associated with the risk of developing sarcomas (*CHEK2, EXT1, EXT2, RB1* and *TP53*), but also in genes where that risk is still unknown (*ERCC2/3, TSC2, RAD50, FANCM* and others) or is emerging (*PALB2, BRCA2*). LOH was evaluated in 8 tumors so far: two presented LOH for variants in *EXT1* and *MITF* and 6 showed no evidence of LOH for variants in *ERCC4, RB1, NF1, PALB2, CHEK2* and *MUTYH*.

Conclusion: Our results highlight a high rate of P/LP variants in CPGs in young Brazilian patients with sarcomas (19.8%) and we expect to collaborate with the definition of effective and adequate screening strategies for these patients.

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P12.104.D Global-screening array for the assessment of homologous recombination deficiency (HRD) in epithelial ovarian cancer

Simon Schnaiter[1], *Esther Schamschula*[1], *Heidelinde Fiegl*[2], *Daniel Reimer*[2], *Alain Zeimet*[2], *Johannes Zschocke*[1], *Katharina Wimmer*[1]

[1]Institut für Humangenetik, Med. Univ. Innsbruck, Innsbruck, Austria, [2]Department für Gynäkologie und Geburtshilfe, Med. Univ. Innsbruck, Innsbruck, Austria.

Introduction: Homologous recombination deficiency (HRD), caused in 40-50% of cases by BRCA1/2 pathogenic variants (PVs), is a predictive biomarker for the response of different tumors to PARP-inhibitor therapy and, in epithelial ovarian cancer (EOC), also considered predictive for sensitivity of platinum-based therapies. Currently, HRD-positivity is mainly assessed by a commercial diagnostic tests with limited transparency of the underlying algorithms.

Methods: To determine HRD positivity we examined genome-wide copy number variation and loss of heterozygosity (LOH) by genotyping 62 ovarian cancers, 22 of which contained a BRCA1/2 PV, using the Global Screening Array (GSA-24 v3.0+Multi-Disease Content; Illumina). Data analysis was performed with Illumina GenomeStudio 1.6.3 (Genotyping Analysis Module) and NxClinical (Biodiscovery, SNP-FASST2-Segmentation Algorithmus) software. For quantification of HRD a LOH-score based on Swisher et al (2017; PMID: 27908594) and an Aneuploidy Normalized Telomeric Imbalance-Score (ANTI-Score, unpublished) were defined.

Results/Conclusion: The group of BRCA1/2-PV samples had significantly higher median scores than BRCA1/2-wildtype samples (LOH-score: 28 vs. 3.6; ANTI-score: 11 vs. 1.5). LOH-score and ANTI-scores were concordant (R2= 0.89). Based on the lowest scores determined in the BRCA1/2-PV samples, we defined the threshold for HRD-positivity as LOH-score ≥14 and/or ANTI-score ≥6. 8/40 BRCA1/2-wildtype samples had scores above the thresholds and 32 below. To determine whether the formers are true HRD positives we are currently testing these samples for other (epi-) genetic aberrations explaining their potential HRD-positivity. Rapid and reliable HRD analysis is possible with a standard genotyping array on DNA from native tumor tissue. We currently evaluate DNA extracted from FFPE-tissue.

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P12.105.A Sequential somatic *HRAS* mutation and gene duplication in a patient with epidermal nevus and rhabdomyosarcoma: further evidence of a two-hit pathogenetic mechanism contributing to oncogenic transformation

Roberta Zuntini[1], *Lucia Pedace*[2], *Evelina Miele*[2], *Stefano Giuseppe Caraffi*[1], *Stefano Gardini*[3], *Elena Ficarelli*[3], *Riccardo Pampena*[3], *Simone Pizzi*[4], *Francesca Clementina Radio*[4], *Angelica Barone*[5], *Simonetta Piana*[6], *Patrizia Bertolini*[5], *Domenico Corradi*[7], *Maria Marinelli*[1], *Alberico Motolese*[3], *Marco Tartaglia*[4,8], *Livia Garavelli*[1,8]

[1]Medical Genetics Unit, AUSL IRCCS Arcispedale Santa Maria Nuova, Reggio Emilia, Italy, [2]Department of Pediatric Hematology/Oncology and Cellular and Gene Therapy, Ospedale Pediatrico Bambino Gesù, IRCCS, Roma, Italy, [3]Dermatology Unit, Azienda USL, IRCCS, Arcispedale Santa Maria Nuova, Reggio Emilia, Italy, [4]Genetics and Rare Diseases Research Division, Ospedale Pediatrico Bambino Gesù, IRCCS, Roma, Italy, [5]Pediatric Hematology Oncology Unit, University Hospital of Parma, Parma, Italy, [6]Pathology Unit, Deptartment of Oncology and Advanced Technologies, Arcispedale S Maria Nuova-IRCCS, Reggio Emilia, Italy, [7]Unit of Pathology, Department of Medicine and Surgery, University of Parma, Parma, Italy, [8], These authors jointly coordinated this work, Italy.

We report a 7 year-old child with diffuse epidermal nevi on the face and head, right upper limb, thorax, left lower limb, arranged according to Blascko's lines, who developed a left paratesticular embryonic rhabdomyosarcoma at 18 months of age. We used NGS custom panel approach to scan the lesions-associated genomic events using blood, buccal brush, fibroblast and rhabdomyosarcoma tissue samples. Parallel sequencing analysis identified a somatic pathogenic gain-of-function variant (c.37G>C, p.Gly13Arg) in the *HRAS* gene in both epidermal nevus and tumor tissues. Variant reads accounted for 33% and 92% of total reads in the nevus and tumor, respectively, supporting the occurrence of a second event involving the gene specifically arising in the latter. The variant was absent in the DNA extracted from the proband's peripheral blood and buccal brush, indicating its postzygotic origin. DNA methylation profiling microarray analysis was performed on the proband's tumor sample, providing a profile that was consistent with the signature characterizing embryonic rhabdomyosarcomas. The analysis also documented a copy number gain of Chromosome 11, pointing out a structural genomic rearrangement resulting in the duplication of the mutated *HRAS* allele as a second hit in the tumor. Somatic activating mutations of the *HRAS* gene are associated with various neoplasms. Our findings are in line with previously collected data documenting the occurrence of gene dosage events involving activating *HRAS* alleles in cancers occurring in patients with Costello syndrome. This diagnosis will permit adoption of screening measures in the patient to detect malignant transformation at early stages.

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P12.106.C Germline Wnt pathway alterations predispose to colorectal hyperplastic polyposis

Isabel Quintana[1], *Mariona Terradas*[1], *Pilar Mur*[1,2], *Gemma Aiza*[1], *Matilde Navarro*[1,2], *Virginia Piñol*[3], *Joan Brunet*[1,2,4], *Gabriel Capellá*[1,2], *Laura Valle*[1,2]

[1]Hereditary Cancer Program, Catalan Institute of Oncology; Oncobell Program, IDIBELL, Hospitalet de Llobregat, Spain, [2]Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Madrid, Spain, [3]Gastroenterology Unit, Hospital Universitario de Girona Dr Josep Trueta, Girona, Spain, [4]Catalan Institute of Oncology, IDIBGi, Girona, Spain.

Extensive efforts have been made to elucidate the inherited factors that predispose to serrated/hyperplastic polyposis (SP), a heterogeneous disease associated with a significant personal and familial CRC risk. Germline *RNF43* pathogenic variants have been causally linked to the disease, explaining <2% of SP cases. We aimed to identify additional inherited risk factors by performing exome sequencing in 44 non-related SP cases followed by a pathway-centered analysis. We selected rare, predicted damaging variants affecting genes involved in pathways or processes relevant in colorectal carcinogenesis and hereditary cancer, including DNA repair, TGF-β and Wnt pathways. Mutational burden analysis comparing the frequency of predicted pathogenic variants in cases vs. controls (gnomAD, non-Finnish Europeans) identified significant differences in Wnt pathway components: allele frequency in cases was 50% (44/88), compared to 36% (42,649/118,190) in controls ($p = 0.007$). Differences were not observed when analyzing DNA repair and TGF-β pathway components. Focused on the genes involved in Wnt signaling, we identified 44 rare, predicted damaging variants in 34 Wnt-related genes. Of the 34 genes, 11 harbored significantly more predicted damaging variants in SP cases than in controls:

CCDC88C, DKK1, DKK4, HECW1, ITPR3, PSMB3, PSMC3, PSME4, RNF43, TLE4 and *WNT9B*. Analysis of the 11 candidate SP genes is currently being performed in further ~100 SP patients, ~100 adenomatous polyposis patients and ~1000 familial/early-onset CRC patients, with the aim of validating our findings in an independent SP series and confirming that the enrichment of Wnt-related variants is exclusive of SP or, at most, of polyposis phenotypes.

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P12.109.A microRNA profile in Bulgarian laryngeal squamous cell carcinoma

Gergana Stancheva[1], *Silva Kyurkchiyan*[1], *Veronika Petkova*[1], *Julian Rangachev*[2], *Vanyo Mitev*[1], *Diana Popova*[2], *Radka Kaneva*[1], *Todor Popov*[2]

[1]*Molecular Medicine Center, Department of Chemistry and Biochemistry, MU-Sofia, Sofia, Bulgaria,* [2]*Department of Ear- Nose and Throat, MU-Sofia; Clinic of Ear- Nose and Throat, UMHAT "Tsarica Yoanna-ISUL", Sofia, Bulgaria.*

Introduction: Laryngeal squamous cell carcinoma (LSCC) is an aggressive malignancy with poor prognosis, which despite modern treatment protocols, novel molecular markers are required to improve survival. The aim of our study was to reveal the specific miRNAs signature in advanced LSCC as well as to investigate some of discovered hypoxic miRNAs in a validation group of samples. *Material and method:* Expression of 2549 miRNA in fresh-frozen tumour materials and adjacent normal tissue was performed in 12 patients, diagnosed with advanced LSCC during primary laryngectomy, by SurePrint G3 Human MiRNA r21 Microarray Kit, 8 × 60K(Agilent Technologies). The normalization of the data was performed by GeneSpringGX software. The expression of miR-21-3p and miR-210-3p was evaluated in a group of 38 fresh frozen laryngeal tumor samples and adjacent normal tissue by qRT-PCR analysis. Statistical analysis was performed using SPSS 17.0.

Results and discussion: Expression levels of 2549 miRNA were assessed, 242 of those were significantly dysregulated (cut-off>2.0 (FC);BH-FDR < 0.05). After the analysis, a subset of 14 miRNAs was selected -8 upregulated (miR-18a-5p, miR-181a-5p, miR-181b-5p, miR-21-3p, miR-24-3p, miR-93-5p, miR-210-3p, miR-1246) and 6 downregulated (miR-140a-3p, miR-145-5p, miR-148a-5p, miR-204-5p, miR-497, miR-874-3p). miR-21-3p and miR-210-3p revealed to be an important and related to pathways of tumour angiogenesis and hypoxia so they were investigated in a validation group of patients. It was confirmed increased expression levels of miR-21-3p and miR-210-3p, respectively 78.94% and 39.47%. The ROC curve analysis showed that miR-21-3p can distinguish laryngeal tumor from normal tissue (AUC = 0.816; 95% CI:0.720-0.917;p = $1.76.10^{-6}$) with sensitivity of 84.2% and specificity of 73.7% whereas miR-210-3p did not. *Acknowledgements:* Grants:13/12/20.12.2017/NSF;D01-285/17.12.2019/MES/Bulgaria.

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P12.110.B Prognostic evidence of LEF1 isoforms in childhood acute lymphoblastic leukemia

Yucel Erbilgin[1], *Ozden Hatirnaz Ng*[2], *Sinem Firtina*[3], *Fulya Kucukcankurt*[1], *Zeynep Karakaş*[4], *Tiraje Celkan*[5], *Sema Aylan Gelen*[6], *Khusan Khodzhaev*[1], *Müge Sayitoglu*[1]

[1]*Aziz Sancar Institute of Experimental Medicine, Istanbul University, Istanbul, Turkey,* [2]*Acibadem Mehmet Ali Aydinlar University, Istanbul, Turkey,* [3]*Istinye University, Faculty of Art and Science, Istanbul, Turkey,* [4]*Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey,* [5]*Istanbul University-Cerrahpasa Faculty of Medicine, Istanbul, Turkey,* [6]*Faculty of Medicine, Kocaeli University, Kocaeli, Turkey.*

Introduction: The lymphoid enhancer factor 1 (LEF1) is a DNA-binding transcription factor that functions in the Wnt signaling pathway. Increased LEF1 activity is associated with progression of several types of cancer including leukemia. Here, we investigated LEF1 isoforms expression and genomic variations in acute lymphoblastic leukemia (ALL).

Methods: LEF1 isoforms expression was evaluated by quantitative real-time PCR in 87 newly diagnosed childhood ALL patients and controls. Moreover, Western blot analysis was performed for detection of LEF1 expression and the hotspot region of LEF1 was screened by deep sequencing.

Results: The LEF1 mRNA expression of B-cell ALL patients was higher than the controls (LEF1-totalp = 0.011, LEF1-long p = 0.026). Moreover, B-ALL samples showing higher total LEF1 expression had significantly shorter relapse-free survival (p = 0.008) and overall survival (p = 0.011). Although, full length LEF1 expression was similar to the controls in T-ALL, 50% (n = 15) of the ALL patients had increased full-length LEF1 protein expression. Imbalance between short and full-length LEF1 isoforms may lead to cell survival in ALL. Beside the LEF1 activation, LEF1 gene variations were rarely observed in our cohort.

Conclusion: The results indicate that the Wnt pathway may have a pathogenic function in a group of ALL patients and high LEF1-total expression might be a marker for shorter relapse free survival time in B-cell ALL.

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P12.111.C Implementation of an integral strategy for personalized medicine based on Duplex Sequencing and cell-free DNA

Pau Rodriguez-Sodupe[1], *Karolina Henryka Czarnecka*[1], *Luis Alberto Pérez-Jurado*[2], *Lluis Armengol Dulcet*[1], *Jairo Rodriguez Lumbiarres*[1]

[1]*qGenomics, Esplugues del Llobregat, Spain,* [2]*Universitat Pompeu Fabra, Barcelona, Spain.*

Ultrasensitive and specific methods for rare allele detection are essential in order to fully exploit the potential of identifying a single genetic variant that might be poorly represented in a biological mixture (liquid biopsy, for instance). In this context, several methods have been described that use unique molecular identifiers (UMIs) to analytically remove NGS errors. Among them, Duplex Sequencing (DS) has been shown to be highly-effective by leveraging the sequence complementarity of the two DNA strands. Nevertheless, the described DS adaptors' production methodology leads to a low ligation efficiency, which hinders their capability to work with limited amounts of input DNA such as cell-free DNA (cfDNA) samples. Moreover, DS needs a much higher sequencing depth and is a costly approach together with large panels.

Here, we have devised an efficient and cost-effective approach to produce DS adapters with a double-stranded 12 bp UMI that can be used with cfDNA inputs as low as 2 ng. This, together with the capacity to efficiently produce mixtures of enrichment probes that are able to deliver very high on-target metrics, are key to any personalized medicine strategy. Within the pediatric oncology context, promising preliminary results demonstrate that we can detect circulating tumor DNA (ctDNA) at frequencies down to one in one thousand with extreme accuracy.

In summary, we are laying the foundations for a robust personalized medicine solution that will allow an extremely accurate detection of ultra rare mutations by using small custom panels that may be strictly personalized in different clinical settings.

P. Rodriguez-Sodupe: A. Employment (full or part-time); Significant; qGenomics. **K. Czarnecka:** A. Employment (full or part-time); Significant; qGenomics. **L. Pérez-Jurado:** A. Employment (full or part-time); Modest; qGenomics. **L. Armengol Dulcet:** A. Employment (full or part-time); Significant; qGenomics. **J. Rodriguez Lumbiarres:** A. Employment (full or part-time); Significant; qGenomics.

P12.112.B combinatorial interactions of curcumin and silymarin against proliferation of human liver and colon cancer cells (in-silico and in-vitro study)

Ahmed Mamdouh Osman[1], *Samah Aly Loutfy*[1,2], *Habeba S. Hosny*[3], *Lina SM Fouda*[3], *Mahmoud ER AbdelRaouf*[3], *Amr M. Ageez*[4], *Sophia Kchouk*[5], *Lamees Hegazy*[5]

[1]National Cancer Institute, Cairo University, Cairo, Egypt, [2]Nanotechnology Research Center (NTRC), British University in Egypt (BUE), Cairo, Egypt, [3]Faculty of Biotechnology, Modern Science and Arts (MSA) University, 6th of October city., Cairo, Egypt, [4]Faculty of Biotechnology, Modern Science and Arts (MSA) University, 6th of October city, Cairo, Egypt, [5]Center for Clinical Pharmacology, Washington University School of Medicine and St. Louis College of Pharmacy, St. Louis, MO, MO, USA.

Background/Aim: Newer approaches are required to control human cancers, natural materials derived from plants offer tremendous advantages. Curcumin purified form of turmeric herb and silymarin which derived from Silybum marianum demonstrated anticancer activities. The synergistic effect of both phytochemicals has been reported against proliferation of some human cancers, therefore, we aimed at evaluating the cytotoxic effect of both materials separately and when mixed to obtain the maximum synergistic effect.

Methods: IC50s for curcumin, silymarin were determined on human liver cancer (huh7) and on human colon cancer cells (HCT116) compared to normal cells (Vero), and when mixed together using MTT. Nature of the relationship was determined by CompuSyn software. Sensitization of cells was performed to determine which one exerted its cytotoxic effect. Molecular docking for materials in BCL2 and EGFR was performed using the Glide package within Schrodinger's Maestro interface to elucidate their anticancer activities.

Results: IC50 of curcumin and silymarin separately were 12µg/ml, 5.4µg/ml, and 7.4µg/ml, 12.9 µg/ml on huh7 and HCT116 respectively, whereas were 30.5 µg/ml and 335 µg/ml, respectively on Vero cells. Such IC50s were markedly reduced upon combination at 0.8, 2.2 and 5 µg/ml on huh7, HCT116 and Vero, respectively. Pre-exposure studies indicated sensitization of curcumin to silymarin on both cancer cells. In silico study demonstrated favourable docking into the binding sites of BCL2 and EGFR proteins.

Conclusion: Combination of curcumin and silymarin at low concentrations reduced the proliferation of human liver and colon cancer cells compared to normal, their apoptotic effect was confirmed by In-silico analysis.

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P12.113.A Extended gene panel testing in lobular breast cancer

Elke M. van Veen[1,2], *D Gareth Evans*[1,2,3,4], *Elaine F. Harkness*[3,5], *Helen J. Byers*[1,2], *Jamie M. Ellingford*[1,2], *Emma R. Woodward*[1,2], *Naomi L. Bowers*[2], *Andrew J. Wallace*[2], *Sacha J. Howell*[3,4,6], *Anthony Howell*[3,4], *Fiona Lalloo*[2], *William G. Newman*[1,2], *Miriam J. Smith*[1,2]

[1]Division of Evolution and Genomic Sciences, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester Academic Health Science Centre, Manchester, United Kingdom, [2]Manchester Centre for Genomic Medicine, Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom, [3]Prevent Breast Cancer Centre, Wythenshawe Hospital Manchester Universities Foundation Trust, Manchester, United Kingdom, [4]Manchester Breast Centre, The Christie NHS Foundation Trust, Manchester, United Kingdom, [5]Division of Informatics, Imaging and Data Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester Academic Health Science Centre, Manchester, United Kingdom, [6]Division of Cancer Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester Academic Health Science Centre, Manchester, United Kingdom.

Purpose: Lobular breast cancer (LBC) accounts for ~15% of breast cancer. Here, we studied the frequency of pathogenic germline variants (PGVs) in an extended panel of genes in women affected with LBC.

Methods: 302 women with LBC and 1567 without breast cancer were tested for *BRCA1/2* PGVs. A subset of 134 LBC affected women who tested negative for *BRCA1/2* PGVs underwent extended screening, including: *ATM, CDH1, CHEK2, NBN, PALB2, PTEN, RAD50, RAD51D* and *TP53*.

Results: 35 PGVs were identified in the group with LBC, of which 22 were in *BRCA1/2*. Ten actionable PGVs were identified in additional genes (4x*ATM*, 1x*CDH1*, 1x*CHEK2*, 2x*PALB2* and 2x*TP53*). Overall, PGVs in four genes conferred a significant increased risk for LBC. Odds ratios (ORs) were: *BRCA1*: OR = 13.17 (95%CI: 2.83-66.38; P = 0.0017), *BRCA2*: OR = 10.33 (95%CI: 4.58-23.95; P < 0.0001), *TP53*: OR = 75.74 (95%CI: 8.72-1098; P = 0.0020) and *ATM:* OR = 8.01 (95%CI: 2.52-29.92; P = 0.0053). We did not detect an increased risk of LBC for *PALB2, CDH1* or *CHEK2*.

Conclusion: The overall PGV detection rate was 11.59%, with similar rates of *BRCA1/2* (7.28%) PGVs as for other actionable PGVs (7.46%), indicating a benefit for extended panel genetic testing in LBC. We also report a previously unrecognised association of pathogenic variants in *ATM* with LBC.

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P12.114.B Liquid biopsy in lung cancer patients shows advantages compared to FFPE tissue mutational analysis

Madli Tamm[1], *Tarmo Annilo*[1], *Kersti Oselin*[2], *Mart Kals*[1,3], *Katrin Keerma*[1,4], *Paula Ann Kivistik*[1], *Miriam Nurm*[1,5], *Margot Saare*[1,6], *Jana Jaal*[7,8], *Neeme Tõnisson*[1,9]

[1]*Estonian Genome Centre, Institute of Genomics, University of Tartu, Tartu, Estonia,* [2]*Department of Chemotherapy, Clinic of Oncology and Haematology, North Estonia Medical Centre, Tallinn, Estonia,* [3]*Institute for Molecular Medicine Finland, FIMM, HiLIFE, University of Helsinki, Helsinki, Finland,* [4]*Tartu University Hospital, Tartu, Estonia,* [5]*Institute of Technology, University of Tartu, Tartu, Estonia,* [6]*Central laboratory, Diagnostic Clinic, East Tallinn Central Hospital, Tallinn, Estonia,* [7]*Haematology and Oncology Clinic, Tartu University Hospital, Tartu, Estonia,* [8]*Institute of Clinical Medicine, University of Tartu, Tartu, Estonia,* [9]*Department of Genetics, United Laboratories, Tartu University Hospital, Tartu, Estonia.*

Introduction: Cancer burden is still a globally growing problem. Early diagnosis and targeted treatment decrease patients' death rate, pain, and treatment expenses. Liquid biopsy can be used for early detection, treatment selection, cancer progression and treatment response monitoring.

Materials and Methods: 106 treatment-naïve advanced stage lung adenocarcinoma patients donated blood samples at baseline and at progression (N = 22). Matched FFPE biopsy samples were available for 75 patients. We set up a 21-amplicon targeted sequencing workflow for analyzing mutations in the EGFR pathway. Genetic findings were compared to patients' clinical profiles.

Results: Higher cfDNA concentration was associated with poorer overall survival (OS). Detectable mutations (variant allele frequency, VAF>0.8%) were in 63 (59%) patients' baseline samples with median VAF 1.13%. Gene-based analyses on the cfDNA revealed that mutations in *ALK* and *EGFR* were positively associated with OS. Considering all analyzed 9 genes, OS was significantly longer for patients with detected mutations in cfDNA. We found that patients with slowly progressing disease carried significantly more mutations in their cfDNA.

Conclusions: Compared to FFPE biopsy material that didn't show any statistically significant correlation, cfDNA has better prognostic properties for predicting patients' OS and disease progression rate. It can be done either by simply measuring the cfDNA concentration or looking deep into somatic mutations. Based on our results we believe that liquid biopsy can mark the new era in cancer treatment. This research was supported by the EU project 2014-2020.4.01.15-0012, and Estonian Research Council PUT736, PUT PRG555 grants.

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P12.115.C Targeted gene panel sequencing in patients with lung cancer

Natalia Brodaczewska, *Hongxiang Liu*

Cambridge University Hospitals NHS Trust, Cambridge, United Kingdom.

In recent years, treatment of lung cancer has been revolutionised by development of agents that target specific variants within the cancer genome, such as the tyrosine kinase inhibitors designed to target mutant EGFR, ALK or ROS1. New therapies targeting variants in other genes are currently under investigation and it is hoped that, as we deepen our understanding of genetic changes underlying lung cancer, more effective treatment strategies can be developed. In many cases, reliable detection of variants can be hindered by low number of tumour cells available for testing in small biopsy and cytology specimens. This study aimed to evaluate the performance of Next Generation Sequencing (NGS) in a routine healthcare setting and examine the profile of mutations detected in 577 lung cancer patients referred for molecular testing over a two-year period. Targeted NGS testing using the Ion AmpliSeq™ Cancer Hotspot panel was performed on DNA extracted from dissected formalin-fixed paraffin embedded (FFPE) tissue specimens. In total, 909 variants were detected in 40 genes, the most commonly mutated genes being *TP53* (51.2%), *KRAS* (39.2%) and *EGFR* (17.2%). Variants in *EGFR* were predominantly in-frame deletions in exon 19 (37%) and L858R (32%). T790M resistance mutation was detected in 6 patients. Other potentially actionable variants were detected in *BRAF* and *ERBB2*. Different mutational profiles were observed for patients with previous tobacco exposure compared to never-smokers. NGS performance on all specimens was high, with 2.5% overall failure rate and reliable detection of *EGFR* variants at variant allele frequency as low as 2%.

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P12.117.A Co-occurrence of hereditary breast-ovarian cancer and Lynch syndromes:case series and clinical implications

Ido Laish[1], *Eitan Friedman*[2], *Gili Levi-Reznick*[3], *Inbal Kedar*[4], *Lior Katz*[5], *Zohar Levi*[6], *Naama Halpern*[7], *Shani Parnasa*[8], *Aasem Abu-Shatya*[9], *Elizabeth Half*[10], ***Yael Goldberg***[4]

[1]*1Gastroenterology Institute, Chaim Sheba Medical Center, Tel Hashomer, Israel,* [2]*Susanne Levy Gertner Oncogenetics Unit, The Danek Gertner Institute of Human Genetics, Chaim Sheba Medical Center, Tel Hashomer, Israel,* [3]*Genetic Institute, Rambam Health Care Campus, Haifa, Israel,* [4]*Raphael Recanati Genetics Institute, Rabin Medical Center, Petah Tikva, Israel,* [5]*5Department of Gastroenterology and Hepatology, Hadassah Medical Center, Jerusalem, Israel,* [6]*Gastroenterology Institute, Rabin Medical Center – Beilinson Hospital, Petah Tikva, Israel,* [7]*7Department of Oncology, Chaim Sheba Medical Center, Tel Hashomer, Israel,* [8]*8Faculty of Medicine, the Hebrew University of Jerusalem, Jerusalem, Israel,* [9]*9Department of Internal Medicine, Carmel Medical Center, Haifa, Israel,* [10]*10Gastroenterology Institute, Rambam Health Care Campus, Haifa, Israel.*

Purpose: Hereditary breast and ovarian cancer syndrome (HBOC) and Lynch syndrome (LS), the most common inherited cancer syndromes, are attributed to a single heterozygous pathogenic variant (PV) in *BRCA1/2* or in a DNA MMR gene, respectively. Little is known about the phenotype in double heterozygotes who carry PVs in both genes.

Methods: Carriers of double PVs in any DNA MMR gene and *BRCA1/2* attending one of three tertiary oncogenetic clinics between 1/2005 and 1/2020 were identified by database search, and their relevant data were retrieved and analyzed.

Results: Eleven double carriers from four seemingly unrelated Ashkenazi Jewish families were evaluated. All carried an Ashkenazi Jewish founder *BRCA* PV, *BRCA2* c.5946delT/c.6174delT (n = 10) or *BRCA1* c.185delAG (n = 1). Four carried the *MSH2* c.1906G>C founder PV, and 3, the *MSH6* c.3984_3987dupGTCA founder PV; 3 patients had the *MSH6* c.3956_3957dup PV. Eight double carriers (73%) had cancer: breast cancer (5 cases, 2 bilateral), melanoma (2 cases), urothelial cancer (2 cases), and colon, endometrial, prostate, squamous cell cancer, glioblastoma, gastric stromal tumor, and lymphoma (1 case each). Six carriers had 1-2 tumors, one had 3 tumors, and one had 5 primary tumors. Age at diagnosis of first tumor was 36-76 years. All carriers met *BRCA1/2* testing criteria, and 3 met the revised Bethesda guidelines.

Conclusions: This case series, supported by the literature, suggests that the phenotype of double *MSH2/6* and *BRCA1/2* carriers is not associated with early disease onset or a more severe phenotype. The findings have implications for improved genetic testing guidelines and treatment strategies.

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P12.118.B Universal immunohistochemistry for Lynch Syndrome: a systematic review and meta-analysis of 58,580 colorectal carcinomas

Ellis L. Eikenboom[1], *Anne-Sophie S. van der Werf-'t Lam*[2], *Mar Rodríguez-Girondo*[2], *Christi J. van Asperen*[2], *Winand N. M. Dinjens*[1], *Robert M. W. Hofstra*[1], *Monique E. van Leerdam*[2,3], *Hans Morreau*[2], *Manon C. W. Spaander*[1], *Anja Wagner*[1], *Maartje Nielsen*[2]

[1]*Erasmus MC, Rotterdam, Netherlands,* [2]*Leiden University Medical Center, Leiden, Netherlands,* [3]*Netherlands Cancer Institute, Amsterdam, Netherlands.*

Introduction: Lynch Syndrome (LS) is a form of hereditary colorectal cancer (CRC), caused by germline variants in DNA mismatch repair (MMR) genes. Currently, many Western countries perform universal immunohistochemistry (IHC) testing on CRC to increase identification of LS patients and their relatives. For a clear understanding of health benefits and costs, data on its outcomes are required: proportions of LS, sporadic MMR-deficient (MMRd) cases, and unexplained MMRd cases.

Materials and methods: Ovid Medline, Embase, and Cochrane CENTRAL were searched for studies reporting on universal MMR IHC, followed by MMR germline analysis, until March 20, 2020. Proportions were calculated, subgroup analyses were performed based on age and diagnostics used, and random effects meta-analyses were conducted. Quality was assessed using the QUADAS-2 tool.

Results: Of 2723 identified articles, 56 studies covering 58,580 CRCs were included. In 6% (95% CI 5%-8%; I^2=96%) MMR deficient protein staining was identified. MMR germline variants were present in 2.0% (95% CI 2%-2%, I^2=92%), ranging from 1.8% to 7.3% based on completeness of diagnostics and age restriction. IHC outcomes were missing in 13%, germline testing was performed in 76% of eligible patients. In seven studies, including 6848 CRCs completing all diagnostic stages, germline variants and biallelic somatic MMR inactivation were found in 3.0% and 1.7%, respectively; 0.6% remained unexplained MMRd.

Conclusions: Complete diagnostics explained MMRd in almost all CRCs and therefore a small number of patients are candidate for multi gene panel testing. These findings are relevant in application of guidelines for testing and surveillance in MMRd CRCs.

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P12.119.C Preliminary evaluation of highly sensitive assessment of microsatellite instability as a tool for cancer risk individualization in Lynch syndrome

Júlia Canet-Hermida*[1], *Fátima Marín**[1], *Núria Dueñas**[1], *Laura Costas*[2], *Víctor Moreno*[3], *Mònica Salinas*[1], *Àngela Velasco*[4], *Paula Peremiquel-Trillas*[2], *Eduard Dorca*[5], *Jordi Ponce*[6], *Laura Cárdenas*[7], *Francisco Rodríguez-Moranta*[8], *Virginia Piñol*[9], *Gemma Mateu*[10], *Maria José Paüles*[5], *August Vidal*[5], *Eugeni López-Bonet*[10], *Xavier Matias-Guiu*[5], *Joan Brunet*[1,4], *Gabriel Capellá*[1], *Marta Pineda*[1]

[1]*Hereditary Cancer Program, Catalan Institute of Oncology, Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), ONCOBELL Program (CIBERONC), L'Hospitalet de Llobregat (Barcelona), Spain,* [2]*Cancer Epidemiology Research Programme, Catalan Institute of Oncology, Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), L'Hospitalet de Llobregat (Barcelona), Spain,* [3]*Cancer Prevention and Control Program, Catalan Institute of Oncology, Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), CIBERESP, L'Hospitalet de Llobregat (Barcelona), Spain,* [4]*Hereditary Cancer Program, Catalan Institute of Oncology, Institut d'Investigació Biomèdica de Girona (IDIBGI), Girona, Spain,* [5]*Pathology Department, Hospital Universitari de Bellvitge, Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), L'Hospitalet de Llobregat (Barcelona), Spain,* [6]*Department of Gynecology and Obstetrics, Hospital Universitari de Bellvitge, Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), L'Hospitalet de Llobregat (Barcelona), Spain,* [7]*Department of Gynecology and Obstetrics, Hospital Universitari Josep Trueta, Institut d'Investigació Biomèdica de Girona (IDIBGI), Girona, Spain,* [8]*Department of Gastroenterology, Hospital Universitari de Bellvitge, Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), L'Hospitalet de Llobregat (Barcelona), Spain,* [9]*Department of Gastroenterology, Hospital Universitari Josep Trueta, Institut d'Investigació Biomèdica de Girona (IDIBGI), Girona, Spain,* [10]*Pathology Department, Hospital Universitari Josep Trueta, Institut d'Investigació Biomèdica de Girona (IDIBGI), Girona, Spain.*

Lynch syndrome (LS) is associated with increased risk of colorectal (CRC) and endometrial (EC) cancers. Despite intensive surveillance, many LS patients will develop tumors at follow-up. There is a need for a more personalized risk assessment. We aim at evaluating the clinical utility of highly sensitive assessment of microsatellite instability (hs-MSI) in target tissues and non-invasive surrogates for the individualization of surveillance in LS carriers.

Endometrial aspirates, clinician-collected cervical Pap brush samples and cervico-vaginal self-samples were obtained from 97 female controls and 78 LS carriers. Colonic mucosa biopsies were collected from 47 control individuals and 80 LS patients. Histological characterization, MMR protein immunohistochemistry (IHC) were performed in target tissues and hs-MSI metrics were calculated in a subset of cases.

High levels of hs-MSI were detected in aspirates from 2 LS patients with EC, in 2/2 with complex hyperplasia and in 6/25 aspirates with histologically normal endometrium, being negative in aspirates from 9 female controls. The presence of MMR-deficient glands correlated with the hs-MSI levels. In non-invasive samples, hs-MSI scores were positive in 2/2 cervical samples and in 1/2 cervico-vaginal self-samples from LS women with EC. Finally, high hs-MSI levels were found in 1/7 colonic polyps, while the presence of MMRd-crypts were identified in colon mucosa from 2/17 LS patients, persisting in a subsequent colonoscopy biopsy of one of them.

Our hs-MSI approach can help in the detection of endometrial and colorectal malignant and premalignant lesions in LS patients. Further analyses are needed to validate its putative clinical usefulness.

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P12.120.D Determination of Lynch syndrome related colorectal cancer based on tumor testing for microsatellite instability, BRAF V600E and MLH1 promoter hypermethylation among Slovene patients

Petra Skerl[1], *Gasper Klancar*[1], *Vida Stegel*[1], *Vita Setrajcic Dragos*[1], *Vesna Vogric*[1], *Alenka Bombac*[1], *Anja Zagozen Klasinc*[1], *Ira Kokovic*[1], *Ana Blatnik*[2], *Ksenija Strojnik*[2], *Marta Banjac*[2], *Mateja Krajc*[2], *Srdjan Novakovic*[1]

[1]*Department of Molecular Diagnostics, Institute of Oncology Ljubljana, Ljubljana, Slovenia,* [2]*Cancer Genetics Clinic, Institute of Oncology Ljubljana, Ljubljana, Slovenia.*

Introduction: Besides family history data and clinicopathological features there are several laboratory-based strategies that help establish diagnosis of Lynch syndrome (LS), including tumor testing for presence of microsatellite instability (MSI), *BRAF* V600E mutation and *MLH1*-promoter-hypermethylation. Our aim was to select LS related colorectal cancer (CRC) patients based on tumor testing. The final confirmation of LS was performed using germline testing.

Materials and Methods: Between 2018 and 2020, 198 CRC patients were included in the study. All together 237 tumors from these patients were tested. For tumor testing, DNA was extracted from FFPE and in-house MSI multiplex PCR method (adopted by Pagin A *et al*., 2013) and/or SALSA MS-MLPA ME011 (including BRAF V600E mutation) was performed. For germline testing, DNA was extracted from blood and NGS sequencing was performed using Nextera_DNA_Library_Preparation_Kit in combination with Illumina's TruSight_Hereditary_Panel. Variants were classified according to ACMG guidelines.

Results: Among 238 CRC tumors a high microsatellite instability (MSI-H) was detected in 29 (12.2%) tumors from 25 patients. Hypermethylation of the *MLH1* promoter was detected in 2 MSI-H patients, and *BRAF* V600E mutation was detected in 2 MSI-H patients. Five patients with MSI-H tumor (2.5%) had a germline pathogenic variant in one of the MMR genes.

Conclusions: Tumor testing for MSI, *BRAF* V600E mutation and *MLH1* promotor hypermethylation is relevant approach for detecting molecular abnormalities related to LS and therefore useful to distinguish suspected LS related CRC from sporadic MMR deficient CRC.

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P12.121.A Long-read RNA-seq identifies allelic loss and aberrant splicing in cancer genes

Vincent Schwenk[1], *Florentine Scharf*[1], *Rafaela M. Leal Silva*[1], *Monika Morak*[1,2], *Verena Steinke-Lange*[1,2], *Elke Holinski-Feder*[1,2], *Julia M. A. Pickl*[1,2], *Dieter A. Wolf*[1]

[1]*MGZ - Medizinisch Genetisches Zentrum München, Munich, Germany,* [2]*Medizinische Klinik und Poliklinik IV, Campus Innenstadt, Klinikum der Universität München, Munich, Germany.*

Introduction: DNA sequence variants can result in allelic imbalances and aberrant splicing leading to cancer predisposition. Identification and interpretation of these variants is challenging, leaving many patients without molecular diagnosis. Five patients with known variants in DNA mismatch repair genes were re-assessed to gauge the diagnostic potential of Oxford Nanopore (ONT) RNA-seq.

Material and Methods: RNA profiles of 123 cancer genes were obtained from patient lymphocyte cultures using Agilent's SureSelectXT capture and ONT cDNA sequencing. Puromycin treatment of lymphocyte cultures was used to block the degradation of aberrant transcripts by nonsense-mediated mRNA decay (NMD).

Results: An average sequencing depth of up to 5,000x was achieved, allowing detailed evaluation of cancer-related transcripts. The heterozygous *MSH2* variant c.1147C>T p.Arg383* led to allelic imbalance with a ratio of 82:17 which was restored to 67:32 by puromycin, indicating an allelic reduction in mRNA expression due to NMD. Monoallelic *MLH1* germline promoter methylation was found to completely abolish mRNA expression from the methylated allele. The *MLH1* variant c.1558+1G>A showed a splicing defect apparent as partial retention of intron 13 in 20% of the transcripts. This fraction increased to 40% after puromycin treatment, suggesting that aberrantly spliced mRNA is subjected to NMD. In-frame skipping of exon 7 in ~50% of transcripts was found for *MSH2* variant c.942+3A>T. Finally, we identified a fusion transcript linking *MLH1* exon 1 to *DCLK3* exons 4-5 caused by a genomic inversion.

Conclusions: Through assessment of allelic mRNA expression and splicing, long-read RNA-seq facilitates variant interpretation and may ultimately increase diagnostic yield.

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P12.122.B Identification of two Lynch syndrome families harboring inherited *MLH1* epimutations

Covadonga Vara[1], *Aida Bujosa*[2], *Júlia Canet*[1], *Consol López*[3], *Mónica Salinas*[1], *Estela Dámaso*[1], *Adriana Lasa*[4], *Conxi Lázaro*[1], *Megan Hitchins*[5], *Joan Brunet*[1,6], *Teresa Ramón y Cajal*[2,3], *Gabriel Capellá*[1], *Marta Pineda*[1]

[1]*Hereditary Cancer Program, Institut Català d'Oncologia – IDIBELL, L'Hospitalet del Llobregat, Spain,* [2]*Servicio de Oncología Médica, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain,* [3]*Unidad de Cáncer Familiar, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain,* [4]*Servicio de Genética, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain,* [5]*Cedars-Sinai Center for Bioinformatics and Functional Genomics, Los Angeles, CA, USA,* [6]*Institut Català d'Oncoloiga- IDIBGI, Girona, Spain.*

Lynch Syndrome is the most common cause of hereditary colorectal and endometrial cancers. Although rare, it can be caused by constitutional *MLH1* epimutations, leading to allele-specific loss of expression due to promoter hypermethylation. *MLH1* epimutations can appear secondary to a genetic alteration in *cis* and following an autosomal dominant pattern of inheritance (secondary epimutations), or as a *de novo* event associated with null or non-mendelian inheritance (primary epimutations). Here, we present two Lynch syndrome families harboring inherited *MLH1* epimutations. Case A is a woman diagnosed with a colorectal adenocarcinoma at age 46, followed by a colorectal adenoma at 48 and multiple myeloma at 50. Case B is a woman with colorectal cancer at 42 years of age (case 7, PMID: 32635641). In both cases, analyses of the colorectal lesions revealed MLH1/PMS2 expression loss, microsatellite instability and *MLH1* methylation. Further MS-MCA and pyrosequencing analyses detected *MLH1* promoter methylation in blood in both cases (~50%). Interestingly, case A's mother, affected by ovarian cancer at age 50, was also an *MLH1* epimutation carrier. Recent analyses of case B's

maternal aunt, diagnosed with multiple CRC at 43 and 85 years of age, showed that she also harbored hemiallelic *MLH1* promoter methylation in blood. The screening of additional relatives of case B is currently being performed. The characterization of inheritance patterns and the identification of causal genetic mechanisms in *cis* in these families will be highly relevant for genetic counseling of epimutation carriers and their relatives.

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P12.123.C Determination of binary protein-protein interaction between PMS2 and a novel germline variant of MLH1 using yeast two-hybrid assay analysis

Eva Jakljevič[1,2], *Tina Zavodnik*[1,3], *Kristina Marton*[1], *Ana Blatnik*[4], *Uroš Petrovič*[1,2]

[1]*Jožef Stefan Institute, Ljubljana, Slovenia,* [2]*Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia,* [3]*Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ljubljana, Slovenia,* [4]*Institute of Oncology, Ljubljana, Slovenia.*

Introduction: Lynch syndrome (LS) is associated with DNA mismatch repair system deficiency, caused by germline pathogenic variants in *MLH1, PMS2, MSH6*, or *MSH2*, or an *EPCAM* deletion. MLH1 and PMS2 proteins form a functional dimer MutLα, the stability of which can be tested using the yeast two-hybrid system (Y2H). In this study, our goal was to determine the pathogenicity of a novel MLH1 in-frame deletion variant MLH1_del746-749: LRG_216t1: c.2236_2247delCTGCCTGATCTA p.(Leu746_Leu749del). This variant has previously been identified in a Slovenian family with confirmed LS and reported as likely pathogenic.

Methods: Protein structure prediction of MLH1 variants were constructed using Phyre and I-TASSER protein modelling tools. Binary protein-protein interactions between PMS2 and variants of MLH1 were investigated using Y2H and 3-amino-1,2,4-triazole (3-AT) gradient. 3-AT increases the stringency of protein-protein interaction Y2H assay by raising the minimum amount of reporter gene expression necessary to enable growth of yeast cells.

Results: The analysis of protein structure models suggested that MLH1_del746-749 cannot interact with PMS2. In the Y2H assay, MLH1_del746-749 interacted with PMS2 in the absence of 3-AT, whereas 30 mM 3-AT prevented its interaction with PMS2, but not the interaction of a non-pathogenic (reference) variant of MLH1.

Conclusion: Based on Y2H results we propose MLH1_del746-749 is pathogenic since its dimer with PMS2 is not stable enough under physiological conditions in human cells. We propose Y2H with 3-AT gradient as an effective and simple semi-quantitatve test for binding affinity between different variants of MLH1 and PMS2, which can aid in establishing the molecular cause of LS.

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P12.124.D Lynch Syndrome - An atypical case of co-occurring *MLH1* promotor hypermethylation and *MLH1* germline likely-pathogenic variant

Fiona Chan Pak Choon[1,2], *Anne-Sophie Chong*[1,2], *Leora Witkowski*[2], *Mona Alameldin*[3], *Gabriela Ghitulescu*[4], *George Chong*[1], *William Foulkes*[1,2,5]

[1]*Lady Davis Institute and Segal Cancer Centre, Jewish General Hospital, Montreal, QC, Canada,* [2]*Department of Human Genetics, McGill University, Montreal, QC, Canada,* [3]*Department of Pathology, Jewish General Hospital, Montreal, QC, Canada,* [4]*Department of Surgery, Jewish General Hospital, Montreal, QC, Canada,* [5]*Department of Medical Genetics and Cancer Research Program, Research Institute of the McGill University Health Centre, Montreal, QC, Canada.*

Lynch Syndrome (LS) is a hereditary disorder caused by germline pathogenic variants in mismatch repair genes (MMR - *MLH1*, *MSH2*, *MSH6* and *PMS2*) that predispose carriers to colorectal cancer. Screening for LS is recommended for all newly-diagnosed colorectal cancer patients. If somatic testing reveals microsatellite instability (MSI) and/or loss of MMR expression via immunohistochemistry (IHC), the tumour samples are further tested for a *BRAF* V600E mutation and *MLH1* promotor hypermethylation - strong indicators of non-inherited colorectal cancer. Only in the absence of those indicators is the germline tested to confirm a LS diagnosis. We report a 45-year-old woman with an ileocecal adenocarcinoma showing MSI and loss of MLH1 and PMS2 by IHC. Sequencing of the tumour and germline revealed a novel *MLH1* germline mutation (c.788A>T), a somatic *MSH6* mutation and a *BRAF* V600E mutation. Interestingly, *MLH1* promotor hypermethylation was also detected in the tumour, but not in the germline. cDNA analysis of the patient's blood showed that the *MLH1* variant causes exons 9 and 10 skipping, which supported our assessment of the variant as likely pathogenic according to the ACMG guidelines. Thus, we diagnosed the patient with LS despite the presence of *MLH1* promotor hypermethylation and *BRAF* V600E mutation in the cecal tumour. Co-occurring *MLH1* promotor hypermethylation and MMR germline mutation cases are rare but do exist. This suggests that decisions on when to test the germline in persons with *MLH1* promotor hypermethylation and a *BRAF* V600E mutation need to be carefully considered. Funded by CIHR grant (FDN-148390) to WF.

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P12.126.B Genome-wide analysis of DNA methylation reveals distinct epigenetically defined subgroups of cutaneous melanoma

Simon Schwendinger[1], *Wolfram Jaschke*[1], *Theresa Walder*[1], *Van Anh Nguyen*[1], *Jürgen Hench*[2], *Stephan Frank*[2], *Johannes Zschocke*[1], *Matthias Schmuth*[1], *Emina Jukic*[1]

[1]*Medical University of Innsbruck, Innsbruck, Austria,* [2]*University Hospital Basel, Basel, Switzerland.*

Introduction: DNA methylation analysis is an emerging method in the diagnosis and prognostication of neoplastic diseases. Aberrant DNA methylation is described as an important contributor in tumorigenesis of cutaneous melanoma. The presented work focuses on the characterization of genetically distinct melanoma samples using microarray-based methylome analysis followed by uniform manifold approximation and projection (UMAP) for dimension reduction.

Materials and Methods: Macrodissected FFPE samples of cutaneous melanoma samples, benign nevi and skin controls were analyzed using a genome-wide DNA methylation array. UMAP was computed on a publicly available computer infrastructure. Melanoma samples were analyzed by NGS with a custom hybridization-capture based sequencing approach investigating

approximately 150 genes recurrently altered in various malignant neoplasms.

Results: DNA methylome analysis by UMAP reveals a segregation of melanoma samples from control epidermis and benign nevi. Cutaneous melanomas can be further divided into two subgroups: One methylation group comprises neoplasms harboring genetic alterations in the MAPK/ERK pathway, i.e. variants of *BRAF*, *RAS*-family genes or *NF1*. The other subgroup includes samples with triple-wildtype genotype, lack of UV-signature and *SOCS1* variants. A third abnormal methylation cluster is suspected to comprise samples with a low tumor cell content due to lower variant allele frequencies of genetic variants and the lack of copy number variants.

Conclusions: The presented work reveals that UMAP dimension reduction of whole-genome methylation analysis data can be utilized to distinguish cutaneous melanoma from benign nevi. Triple-wildtype melanomas seem to present a unique methylation pattern and can be clearly differentiated from MAPK/ERK pathway altered melanoma cases.

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P12.127.C Germline pathogenic variants in DNA repair genes predispose to MPM in asbestos exposed patients

Marta La Vecchia[1], *Marika Sculco*[1], *Anna Aspesi*[1], *Michela G. Clavenna*[1], *Elisabetta Casalone*[2], *Alessandra Allione*[2], *Federica Grosso*[3], *Roberta Libener*[4], *Alberto Muzio*[5], *Ottavio Rena*[6], *Guido Baietto*[6], *Sara Parini*[6], *Renzo Boldorini*[7], *Enrica Migliore*[8], *Dario Mirabelli*[8,9], *Corrado Magnani*[10], *Daniela Ferrante*[10], *Giuseppe Matullo*[2,9,11], *Irma Dianzani*[1,9]

[1]*Department of Health Sciences, Università del Piemonte Orientale, Novara, Italy,* [2]*Department of Medical Sciences, Università di Torino, Torino, Italy,* [3]*Mesothelioma Unit, AO SS. Antonio e Biagio e Cesare Arrigo, Alessandria, Italy,* [4]*Pathology Unit, AO SS. Antonio e Biagio e Cesare Arrigo, Alessandria, Italy,* [5]*Division of Medical Oncology, Ospedale Santo Spirito, Casale Monferrato, Alessandria, Italy,* [6]*Thoracic Surgery Unit, AOU Maggiore della Carità, Novara, Italy,* [7]*Department of Health Sciences, Section of Pathological Anatomy, Università del Piemonte Orientale, Novara, Italy,* [8]*Unit of Cancer Epidemiology, CPO-Piemonte and Università di Torino, Torino, Italy,* [9]*Interdepartmental Center for Studies on Asbestos and other Toxic Particulates "G. Scansetti", Università di Torino, Torino, Italy,* [10]*Department of Translational Medicine, Unit of Medical Statistics, Università del Piemonte Orientale and Cancer Epidemiology, CPO Piemonte, Novara, Italy,* [11]*Medical Genetics Unit, AOU Città della Salute e della Scienza di Torino, Torino, Italy.*

Introduction: Malignant pleural mesothelioma (MPM) is a tumor associated to asbestos exposure. We and others found that approximately 10% of MPM patients carry a germline pathogenic variant (PV) in DNA repair genes.

Materials and Methods: To further extend these data, a total of 206 MPM patients (93 from a previous study, 113 new) were screened by targeted-NGS for germline PVs in cancer-predisposing genes. Six further patients with family history of mesothelioma were analyzed by Sanger sequencing of *BAP1* and *CDKN2A*. Life-long cumulative asbestos exposure was available for 203/212 patients.

Results: We identified 18 PVs in 17/206 patients (8.25%), most of them (14 PVs in 13 patients) were found in genes involved in the DNA repair pathway (i.e. *ATM, BRCA1, BRCA2, BRIP1, CHEK2, FANCC, FANCF, FANCI, PALB2, PMS1, SLX4, XPC*). PVs in *BAP1* and *CDKN2A* were identified in five and one patients with family history of mesothelioma, respectively. Carriers of PVs in DNA repair genes (18/212 patients) showed a statistically significant lower asbestos exposure than non-mutated patients ($p = 0.0001$).

Conclusions: These data suggest that patients with germline mutations in DNA repair genes are less proficient at repairing the DNA damage induced by asbestos and show increased susceptibility to asbestos-induced MPM. The identification of a subset of patients carrying PVs in DNA repair genes may distinguish patients who can benefit from drugs that induce synthetic lethality. **Grants:** HeRMes project (compensation to MPM patients of Casale Monferrato) (ID, CM); AIRC2018-IG21390 (GM)

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P12.128.D A novel germline pathogenic variant *MET* c.3389T>C p.(Leu1130Ser) identified in french population

Molka SEBAI[1], *Marie FERNANDES*[2], *Sandrine M. CAPUTO*[3], *Virginie VERKARRE*[4,5], *Fanny REINHART*[4], *Séverine ADAMS*[1], *Christine MAUGARD*[6], *Olivier CARON*[7], *Marine GUILLAUD-BATAILLE*[1], *Pascaline BERTHET*[8,5], *Yves-Jean J. BIGNON*[9,5], *Jean CHIESA*[10], *Thierry FREBOURG*[11,5], *Sophie GIRAUD*[12,5], *Sophie LEJEUNE*[13], *Jean-Marc M. LIMACHER*[14], *Antoine DE PAUW*[3,15], *Dominique STOPPA-LYONNET*[3,16], *Hélène ZATTARA-CANNONI*[17], *Sophie DEVEAUX*[5], *Rosette LIDEREAU*[3], *Stéphane RICHARD*[5,18], *David TULASNE*[2], ***Etienne ROULEAU***[1]

[1]*Department of Medical Biology and Pathology, Cancer Genetics Laboratory, Gustave Roussy, Villejuif, France,* [2]*University of Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, UMR9020 - U 1277 - CANTHER - Cancer Heterogeneity, Plasticity and Resistance to Therapies, Lille, France,* [3]*Department of Genetics, Institut Curie, Paris, France,* [4]*Department of Pathology, Georges Pompidou European Hospital, Assistance Publique Hôpitaux de Paris, Paris, France,* [5]*French National Network for Rare Cancers in Adults PREDIR labelled by INCa, AP-HP, Hôpital Bicêtre, Kremlin-Bicêtre, France,* [6]*Department of molecular oncogenetics, Hôpitaux Universitaires de Strasbourg, Strasbourg, France,* [7]*Department of Medical Oncogenetics, Gustave Roussy, Villejuif, France,* [8]*Oncologenetics Department, Centre François Baclesse, Caen, France,* [9]*Oncologenetics Department, Centre Jean-Perrin, BP 392, Clermont-Ferrand, France,* [10]*Department of Cytogenetics, Nimes University Hospital, Nîmes, France,* [11]*Department of Genetics, Rouen University Hospital, Rouen, France,* [12]*Genetics Department, Hospices Civils de LYON, Lyon, France,* [13]*Department of genetics, CHRU Lille, Lille, France,* [14]*Genetics Department, Hôpitaux civils de Colmar, COLMAR, France,* [15]*Paris Sciences Lettres Research University, Paris, France,* [16]*INSERM U830, Institut Curie Paris, Paris, France ; Paris-University, Paris, France,* [17]*Department of Genetics, Hôpital de la Timone Enfants, Marseille, France,* [18]*EPHE, PSL University, UMR 9019 CNRS, Paris-Saclay University, Gustave Roussy, Villejuif, France.*

Introduction: *MET* germline oncogenic variants were described in families with bilateral and multifocal papillary type 1 carcinoma (PRCC1). To date, only ten germline *MET* pathogenic variants were reported in the literature. All of them were missense variants of the tyrosine kinase domain. A somatic non-random duplication of chromosome 7 harboring *MET* pathogenic variant was described as a cytogenetic condition for tumorigenesis. Herein, we provide arguments for the classification of *MET* c.3389T>C p.(Leu1130Ser) as a new germline pathogenic variant.

Methods: *MET* p.(Leu1130Ser) (NM_001127500.2) was identified in four index-cases among a French cohort of 158 patients with PRCC1

tumors and accessible *MET* molecular screening. *MET* p.(Leu1130Ser) was first described as a likely pathogenic variant. Familial and clinical informations were collected from medical records. For two index-cases, tumor profiles were documented using array CGH. ERK phosphorylation of *MET* p.(Leu1130Ser) was studied after a transient transfection of 24 hours. Focus formations assays were performed on NIH3T3 cell lines transfected with *MET* p.(Leu1130Ser).

Results: Tumoral features were suggestive of a *MET* genetic predisposition: PRCC1 tumors were bilateral (4/4) and multifocal (3/4). A duplication of chromosome 7 harboring *MET* p.(Leu1130Ser) was found in array CGH. *MET* c.3389T>C p.(Leu1130Ser) was not reported in databases. The Leucine in codon 1130 was highly conserved between species and between tyrosine kinases. *MET* p.(Leu1130Ser) caused a constitutive phosphorylation of ERK protein and induced an abnormal focus formation when transfected into NIH3T3 cell.

Conclusion: Based on the above arguments, *MET* p.(Leu1130-Ser) was classified as a pathogenic variant according to the ACMG recommendations.

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P12.129.A Specific phenotype of germline *MET* mutations in papillary renal cell carcinoma type 1: about a large french series of 158 patients

Molka SEBAI[1], *David TULASNE*[2], *Sandrine M. CAPUTO*[3], *Virginie VERKARRE*[4,5], *Marie FERNANDES*[2], *Fanny REINHART*[4], *Séverine ADAMS*[1], *Christine MAUGARD*[6], *Olivier CARON*[7], *Marine GUILLAUD-BATAILLE*[1], *Pascaline BERTHET*[8,5], *Yves-Jean J. BIGNON*[9,5], *Jean CHIESA*[10], *Thierry FREBOURG*[11,5], *Sophie GIRAUD*[12,5], *Sophie LEJEUNE*[13], *Jean-Marc M. LIMACHER*[14], *Antoine DE PAUW*[3,15], *Dominique STOPPA-LYONNET*[3,16], *Hélène ZATTARA-CANNONI*[17], *Sophie DEVEAUX*[5], *Rosette LIDEREAU*[3], *Stéphane RICHARD*[5,18], *Etienne ROULEAU*[1]

[1]*Department of Medical Biology and Pathology, Cancer Genetics Laboratory, Gustave Roussy, Villejuif, France,* [2]*University of Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, UMR9020 - U 1277 - CANTHER - Cancer Heterogeneity, Plasticity and Resistance to Therapies, Lille, France,* [3]*Department of Genetics, Institut Curie, Paris, France,* [4]*Department of Pathology, Georges Pompidou European Hospital, Assistance Publique Hôpitaux de Paris, Paris, France,* [5]*French National Network for Rare Cancers in Adults PREDIR labelled by INCa, AP-HP, Hôpital Bicêtre, Kremlin-Bicêtre, France,* [6]*Department of molecular oncogenetics, Hôpitaux Universitaires de Strasbourg, Strasbourg, France,* [7]*Department of Medical Oncogenetics, Gustave Roussy, Villejuif, France,* [8]*Oncologenetics Department, Centre François Baclesse, Caen, France,* [9]*Oncologenetics Department, Centre Jean-Perrin, BP 392, Clermont-Ferrand, France,* [10]*Department of Cytogenetics, Nimes University Hospital, Nîmes, France,* [11]*Department of Genetics, Rouen University Hospital, Rouen, France,* [12]*Genetics Department, Hospices Civils de LYON, Lyon, France,* [13]*Department of genetics, CHRU Lille, Lille, France,* [14]*Genetics Department, Hôpitaux civils de Colmar, COLMAR, France,* [15]*Paris Sciences Lettres Research University, Paris, France,* [16]*INSERM U830, Institut Curie Paris, Paris, France,* [17]*Department of Genetics, Hôpital de la Timone Enfants, Marseille, France,* [18]*EPHE, PSL University, UMR 9019 CNRS, Paris-Saclay University, Gustave Roussy, Villejuif, France.*

Introduction: Activating pathogenic variants of *MET* gene were identified in papillary renal cell carcinoma type 1 (PRCC1) with characteristic bilateral and multifocal PRCC1 tumors. Due to the rarity of *MET* deleterious alterations, we decided to study the incidence of *MET* germline mutations in the French population and to describe the specific phenotype of mutation carriers.

Methods: We reviewed the medical and molecular records of 158 patients with PRCC1 tumors and screened for *MET* germline variants (153 index-cases and five relatives).

Results: *MET* pathogenic variant rate among index-cases was 10.4% (16/153) with 37,5% of familial PRCC1 and 3.3% of sporadic PRCC1. Genetic screening showed a variant of uncertain significance in 7.9% of index-cases (12/153) and did not identify a deleterious variant in 81.7% of index-cases (125/153). Four different germline *MET* pathogenic variants were highlighted and three of them were already reported (*MET* p.(His1112Arg); *MET* p.(Val1238Ile); *MET* p.(Tyr1248Cys)). *MET* c.3389T>C (p.(Leu1130Ser)) was a novel missense variant within the tyrosine kinase domain, identified in four families. A strong genotype-phenotype correlation was found among *MET* mutated cases characterized by PRCC1 tumors with mainly bilateral (82.3%) and multifocal (85.8%) onset. No significant difference was observed in the age of diagnosis and in gender between *MET* mutation carriers and cases with wild-type *MET*.

Conclusion: Our results will help to better identify families with genetic predisposition to PRCC1 tumors and support the fact that the clinical presentation is a strong argument to be considered to classify novel *MET* gene missense variants especially when functional assays aren't accessible.

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P12.132.D Comprehensive analysis of correlations in expression of miRNA genes and immune checkpoint genes in bladder cancer cells

Przemyslaw A. Stempor[1], *Paula Dobosz*[2]

[1]*SmartImmune Ltd, Accelerate Cambridge, University of Cambridge Judge Business School, Cambridge, United Kingdom,* [2]*Department of Hematology, Oncology and Internal Medicine, Medical University of Warsaw, Warsaw, Poland.*

Personalised medicine is the future and hope for many patients, including those with cancers. Developing personalised therapeutics and associated diagnostics requires interdisciplinary teams, including oncologists, geneticists, immunologists, pathologists etc. Bladder cancer (BC) is a frequent neoplasm, with high lethality and lacking modern, advanced therapeutic modalities, such as immunotherapy. Early detection, as well as rapid, well selected treatment, are key factors leading to good prognosis. MicroRNA mediated gene regulation is a promising area of development for new diagnostic and therapeutic methods, crucial for better prospects for patients with bladder cancer. MicroRNAs are involved in bladder cancer pathogenesis, proliferation, control and response to treatment.

We performed a correlation based analysis of miRNA and gene expression data in bladder cancer (BLCA) TCGA dataset. We identified 27 miRNAs hits with opposite expression profile to genes involved in immune response in bladder cancer, and 24 miRNAs hits with similar expression profile. Previous studies linking these microRNAs to function in bladder cancer, and asses if they are good candidates for personalised medicine therapeutics and diagnostics. These functions include regulation of gene expression, interplay with transcription factors, response to

treatment, apoptosis, cell proliferation and angiogenesis, initiation and development of cancer, genome instability, and tumour-associated inflammatory reaction.

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P12.133.A Somatic non-epigenetic mismatch repair gene aberrations underly most mismatch repair-deficient Lynch-like tumors

Lisa Elze[1], *Arjen R. Mensenkamp*[1], *Iris D. Nagtegaal*[1], *Wendy A. G. van Zelst-Stams*[1], *Brigit Wapstra*[1], *Charlotte J. Dommering*[2], *Mirjam M. de Jong*[3], *Fonnet E. Bleeker*[4], *Edward M. Leter*[5], *Tom G. W. Letteboer*[6], *Maartje Nielsen*[7], *Rachel S. van der Post*[1], *Nicoline Hoogerbrugge*[1], *Richarda M. de Voer*[1], *Marjolijn J. L. Ligtenberg*[1]

[1]*Radboud university medical center, Nijmegen, Netherlands,* [2]*Amsterdam University Medical Centers, Amsterdam, Netherlands,* [3]*University Medical Center Groningen, Groningen, Netherlands,* [4]*The Netherlands Cancer Institute, Amsterdam, Netherlands,* [5]*Maastricht University Medical Center+, Maastricht, Netherlands,* [6]*University Medical Center Utrecht, Utrecht, Netherlands,* [7]*Leiden University Medical Center, Leiden, Netherlands.*

Introduction: Individuals with Lynch syndrome have a pathogenic germline variant affecting one of the mismatch repair (MMR) genes (*MLH1, MSH2, MSH6* or *PMS2*) and are often recognized by MMR-deficient (dMMR) colorectal or endometrial cancers. The main cause of dMMR is somatic *MLH1*-promoter hypermethylation. dMMR tumors without a germline variant or *MLH1*-promoter hypermethylation (Lynch-like) may have two somatic non-epigenetic MMR aberrations (somatic dMMR). We investigated the cause of dMMR in patients that were referred for diagnostic testing for Lynch syndrome with excluded *MLH1*-promoter hypermethylation.

Materials and Methods: The prevalence of germline and somatic dMMR was analyzed in a cohort of 304 consecutive patients diagnosed below age 70 with dMMR colorectal or endometrium cancer without *MLH1*-promoter methylation. The prevalence of somatic dMMR was also measured in a cohort tested negative for a pathogenic germline variant and *MLH1*-promoter methylation ($n = 125$).

Results: The incidence of germline pathogenic variants and somatic dMMR was 35 versus 51% for *MLH1*-, 43% versus 42% for *MSH2*-, 76% versus 11% for *MSH6*- and 74% versus 9% for *PMS2*-deficient tumors without *MLH1*-promoter methylation. Somatic dMMR was associated with an higher age at diagnosis than Lynch syndrome (52 versus 48 years, $P < 0.01$). Overall somatic dMMR was detected in 87.3% of dMMR tumors without germline MMR gene variants or *MLH1*-promoter methylation.

Conclusion: Especially MLH1- and MSH2-deficient tumors without *MLH1*-promoter methylation are often not due to Lynch syndrome, but have two somatic MMR aberrations. Somatic MMR testing significantly reduces the amount of patients that remain uncertain about their genetic susceptibility to Lynch syndrome.

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P12.134.B Frequency of *de novo* and mosaic *STK11* variants in Peutz-Jeghers syndrome

Albain Chansavang[1,2], *Marion Dhooge*[2,3], *Aurélie Toussaint*[1], *Véronique Duchossoy*[1], *Virginie Benoit*[1], *Joëlle Cohen*[1], *Chrystelle Colas*[4], *Hélène Delhomelle*[4], *Pierre Laurent-Puig*[5], *Aziz Zaanan*[6], *Solenne Farelly*[3], *Camille Tlemsani*[2], *Romain Coriat*[3], *Audrey Briand-Suleau*[1,2], *Béatrice Parfait*[1,2], *Eric Pasmant*[1,2], *Nadim Hamzaoui*[1,2]

[1]*Service de Génétique et Biologie Moléculaires, Hôpital Cochin, APHP. Centre Université de Paris, Paris, France,* [2]*Institut Cochin, Inserm U1016, CNRS UMR8104, Université de Paris, CARPEM, Paris, France,* [3]*Service de Gastroentérologie et Endoscopie, Hôpital Cochin, APHP. Centre Université de Paris, Paris, France,* [4]*Département de génétique, Institut Curie, Université de Recherche Paris Sciences et Lettres, Paris, France,* [5]*Service de Biochimie, Pharmacologie et Biologie Moléculaire, Hôpital Européen Georges-Pompidou, AP-HP, Université Paris Descartes, Paris, France,* [6]*Service de Gastroentérologie et Oncologie Digestive, Hôpital Européen Georges-Pompidou, AP-HP, Université Paris Descartes, Paris, France.*

Peutz-Jeghers syndrome (PJS) (MIM 175200) is an autosomal dominant disease caused by pathogenic variants in the *STK11* gene (alias *LKB1*). PJS is characterized by mucocutaneous pigmentation and hamartomatous polyps, predominantly affecting the small intestine. In adulthood, PJS patients face an increased risk of different cancers. Previous studies showed that more than half of cases are sporadic but the exact proportion of *de novo* cases is still unknown. Among the *de novo* cases, mosaicism could explain the typical PJS cases for which no pathogenic variant is identified in the *STK11* gene. The aim of this work was to evaluate the proportion of *de novo* and mosaic cases in a PJS patient cohort. We analyzed the *STK11* gene using deep targeted sequencing in 84 index cases. A *STK11* pathogenic variant was identified in 87% (73/84) of patients. Sporadic cases were observed in 58% (49/84) of cases, of whom the analysis of 21 trios proved the cases to be *de novo*; 11/84 of cases (13%) were mosaicism with a [14%;37%] variant allele frequency range, suggesting an early post-zygotic event. This study performed on a large series of PJS cases allowed estimating the *de novo* mutations to at least 30%. Considering the medical impact of *STK11* mutation identification, we underlined the importance of appropriate and sensitive techniques to allow the detection of low frequency mosaicism in PJS.

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P12.135.C Mosaic TP53 mutation in a patient with familial and personal history of breast, gastric and bowel cancers

Emanuele Micaglio[1], *Federico Romani*[1], *Michelle Monasky*[1], *Paola Carrera*[2], *Monica Zanussi*[2], *Giorgio Nevio Casari*[2], *Filippo Martinelli Boneschi*[3], *Silvia Presi*[2], *Carlo Pappone*[1]

[1]*IRCCS Policlinico San Donato, San Donato Milanese, Italy,* [2]*IRCCS San Raffaele Hospital, Milan, Italy,* [3]*IRCCS Ospedale Maggiore Policlinico, Milan, Italy.*

Li Fraumeni syndrome is a cancer predisposition phenotype with a high risk of either early onset or adult onset malignances. Li Fraumeni syndrome is often caused by heterozygous germline mutations in *TP53* gene although some cases of mosaicism have been published to date. We describe a three - generation family characterized by history of breast, gastric and bowel cancer, all histologically confirmed. The proband is a 79 years old woman affected by a triple negative ductal breast carcinoma with onset at 44 years old followed by gastric adenocarcinoma at 73 years old and by colon cancer at 75 years old. The proband came to our attention because

her 51 years old daughter required pre-symptomatic genetic counselling. The genetic testing performed in the proband after a careful genetic counselling through analysis of genomic DNA extracted from peripheral blood, demonstrated the c.743G>C heterozygous mutation in *TP53* gene. The sequencing data were consistent with a mosaicism level of about 20%, confirmed by both the absence of aforementioned mutation in proband's saliva extracted DNA and the presence of the same mutation in two different histological samples (gastric and colon cancers) which are conserved elsewhere. The segregation analysis excluded the possibility of germline mosaicism as proband's three sons are negative for the maternal *TP53* mutation. This case highlights the relevance of mosaicism even for late onset cancers and the importance of the teamwork between oncology, anatomopathology and clinical genetics specialists in a modern facility.

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P12.137.A A preliminary analysis of two mitochondrial poly-C tracts in sporadic breast cancer of Sri Lankan ethnicities

Joanne T. Kotelawala[1], *Kamani H. Tennekoon*[1], *Ruwandi Ranasinghe*[1], *H. A. C. I. K. Rodrigo*[1], *G K. S. De Silva*[2], *W A. H. A. Perera*[3], *N. Yoganathan*[3], *M. R. S. Manatunga*[3], *D. M. A. S. Dissanayake*[4], *N. Joseph*[4,5], *C. Rajasooriyar*[4,5], *K Indranath*[4,5]

[1]*Institute of Biochemistry, Molecular Biology and Biotechnology, Colombo 03, Sri Lanka,* [2]*National Cancer Institute, Maharagama, Sri Lanka,* [3]*Kandy Teaching Hospital, Kandy, Sri Lanka,* [4]*Jaffna Teaching Hospital, Jaffna, Sri Lanka,* [5]*Tellippalai Base Hospital, Jaffna, Sri Lanka.*

Introduction: Breast cancer remains the most common cancer among women accounting for nearly 25% of cancers diagnosed. Prior studies have shown two mtDNA poly-C tracts located in the non-coding region (16184-16189 and 303-315) are associated with diseases such as cancer.

Methods: mtDNA non-coding region was studied in newly diagnosed sporadic breast cancer patients and healthy controls among Sri Lankan Tamil and Muslim ethnicities (N = 30 pairs each) of the Sri Lankan population. The non-coding region was amplified using two primer sets and the DNA sequence was obtained using Sanger sequencing.

Results: Here we report variations in mtDNA regions 305-310 bp and 16184-16189 bp observed in the present study with our previously published data for Sinhalese ethnicity (N = 63 pairs of patient and controls; https://doi.org/10.3892/br.2020.1292) used for comparison.

Variation	Sri Lankan Tamil		Muslim	*Sinhalese	
	Patients % (n = 30)	Controls % (n = 30)	Patients % (n = 30)	Controls % (n = 30)	Patients % (n = 63)	Controls % (n = 63)
305-310						
310T>C	3.3	0	0	0	4.8	9.5
309 C ins	46.7	46.7	46.7	33.3	33.3	41.3
315 C ins	86.7	90.0	100	83.3	81.0	85.7
16184-16189						
16184C>T	5.7	0	3.3	3.3	1.6	3.17
16187C>T	3.3	3.3	0	0	3.2	1.59
16188C>T	3.3	0	0	0	0	1.59
16189T>C	23.3	3.3	10.0	3.3	17.5	27.0

*Kotelawala et al, March 2020 **Conclusion**This data shows that variations such as 310T>C identified as a risk factor in other populations is not associated with breast cancer in Sri Lankan ethnic groups. However, variations in the 16184-16189 region were seen to be more inclined towards the formation of a poly-C tract suggesting possible metabolic alterations, which could constitute a risk factor of breast cancer. Further studies need to be carried out to identify if these variations are associated with mitochondrial copy number variations and/or other metabolic factors. This study is funded by the National Science Foundation of Sri Lanka (NSF/SCH/2016/04)

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P12.138.B Evaluation of Plasma Cell Molecular Cytogenetic Findings of Myeloma Patients: One-Year Single-Center Experience

Ibrahim Kaplan, ***Hande Nur Cesur Baltaci****, Sule Altiner, Sadiye Ekinci, Nedime Arzu Vicdan, Halil Gürhan Karabulut, Timur Tuncali, Hatice Ilgin Ruhi, Nüket Yürür Kutlay*

Department of Medical Genetics, School of Medicine, Ankara University, Ankara, Turkey.

Introduction: Multiple myeloma (MM) is a type of plasma cell dyscrasia and the second most common hematological malignancy of adulthood. Malignant plasma cells accumulate in bone marrow leading to bone marrow failure, also in extramedullary sites. During the evaluation of myeloma patients, besides other laboratory examinations, cytogenetic and molecular cytogenetic (FISH) studies of plasma cells and/or bone marrow are especially important for diagnosis, follow-up and providing exact treatment. Here, we present our cytogenetic and molecular cytogenetic results of 93 plasma cell samples from 86 patients, during 2020.

Materials and Methods: We evaluated our FISH results of CD138 (+) plasma cells separated from bone marrow aspirates. In daily practice, we perform FISH analysis for monosomy 7, trisomy 8, 13q deletion, 17p deletion, t(4;14), t(11;14) and if possible, *IGH* and *CCND1* gene rearrangements. In our targeted examinations, atypical results are widely detected that could be seen by routine FISH probes.

Results and Conclusions: According to our results, in 8 of 93 samples (8,6%) trisomy 8; in 43 of 93 samples (46%) (38 of them large deletion or monosomy 13) deletion of 13q; in 13 of 93 samples (13%) loss of *TP53* were detected, whereas monosomy 7 in none. t(11;14) and t(4;14) were detected in 6% and 5% of samples whereas *IGH* gene rearrangements without these translocations were common: in FISH analysis, any type of abnormal pattern was detected with a rate of 53,7% for both fusion probes. All abnormal patterns related to all probes were exclusively discussed.

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P12.139.C Mitochondrial mutational spectrum in human cancers is sensitive to cellular hypoxia

Alina G. Mikhailova[1,2], *Polina Lisitsa*[1], *Alina A. Mikhailova*[1,3], *Kristina Ushakova*[1], *Evgeny Tretiakov*[1,4], *Andrey Yurchenko*[5], *Vsevolod Makeev*[2], *Dmitrii Knorre*[6,7], *Sergey Nikolaev*[5], *Ilia Mazunin*[8,9], *Jacques Fellay*[10], *Konstantin Khrapko*[11], *Konstantin Gunbin*[1], *Konstantin Popadin*[1,10]

[1]Immanuel Kant Baltic Federal University, Kaliningrad, Russian Federation, [2]Vavilov Institute of General Genetics RAS, Moscow, Russian Federation, [3]University of Münster, Münster, Germany, [4]Medical University of Vienna, Vienna, Austria, [5]Université Paris Saclay, Villejuif, France, [6]Moscow State University, Moscow, Russian Federation, [7]Sechenov First Moscow State Medical University, Moscow, Russian Federation, [8]Skolkovo Institute of Science and Technology, Moscow, Russian Federation, [9]Fomin Women's Health Clinic, Moscow, Russian Federation, [10]Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland, [11]Northeastern University, Boston, MA, USA.

The mutational spectrum of the mitochondrial genome (mtDNA) may be sensitive to the oxidative damage since mitochondria maintain the oxidative metabolism. Recently we have shown that the frequency of $A_H>G_H$ (heavy strand notation) substitutions in mtDNA is positively correlated with cellular and organismal longevity (https://doi.org/10.1101/589168). We have shown that somatic $A_H>G_H$ substitutions are more frequent at earlier stages of tumorigenesis and in cancers derived from slow-replicating tissues. The logic behind this finding was that long lived and slow-dividing cells have a rich aerobic environment, permitting a high oxidative metabolism, while short-lived fastly-dividing cells can run out of oxygen ending up in hypoxic conditions. To validate our hypothesis that mtDNA mutational spectrum is sensitive to hypoxia we tested mtDNA mutation rate and spectra in cancer samples with different levels of aerobic metabolism, ranging from normoxia to hypoxia. Using a collection of somatic mtDNA mutations and hypoxia scores derived for thousands individual cancer samples in the framework of the ICGC/TCGA Pan-Cancer Analysis of Whole Genomes (PCAWG) consortium we observed that indeed mtDNA mutations depend on the level of hypoxia. Firstly, the fraction of $A_H>G_H$ is decreased in highly hypoxic cancers. Secondly, the total mtDNA mutation rate is lower in hypoxic cancers (mainly due to drop in $A_H>G_H$). Altogether, we suggest that $A_H>G_H$ substitutions are sensitive to oxidative damage and thus can be a new marker of the redox stress in mtDNA. This work is supported by Russian Science Foundation № 21-75-20143.

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P12.140.D *MUTYH*-associated polyposis in a cohort of Slovenian patients with adenomatous polyposis

Ksenija Strojnik[1], *Iva Opalic*[2], *Mateja Krajc*[1], *Marta Banjac*[1], *Vida Stegel*[3], *Petra Skerl*[3], *Vita Setrajcic Dragos*[3], *Gasper Klancar*[3], *Srdjan Novakovic*[3], *Ana Blatnik*[1]

[1]Cancer Genetic Clinic, Institute of Oncology, Ljubljana, Slovenia, [2]University Medical Centre Maribor, Maribor, Slovenia, [3]Department of Molecular Diagnostics, Institute of Oncology, Ljubljana, Slovenia.

Introduction: *MUTYH*-associated polyposis (MAP) is an autosomal recessive polyposis syndrome caused by biallelic pathogenic variants (PVs) in *MUTYH* gene. It is characterised by multiple colorectal adenomas and high risk of colorectal cancer. Data on extracolonic manifestations of MAP is limited.

Methods: We performed a retrospective analysis of 72 polyposis patients with ≥10 colorectal adenomas referred to our institution for germline genetic testing with NGS panels since 2015.

Results: In 4/72 (5.6%) polyposis patients, all female, biallelic PV in *MUTYH* gene were identified; clinical characteristics are detailed in the table bellow.

Conclusions: We observed 5 different PV in MUTYH gene in our cohort of MAP patients, all of them previously reported in Caucasians. All of our patients had extracolonic tumours.

No.	PV in *MUTYH* (LRG_220t1)	Colorectal polyps (age)	Colorectal cancer (age)	Personal history of other tumours (age)	Family history of cancer (age)	Family history of polyposis
1.	c.536A>G p. (Tyr179Cys) c.734G>A p. (Arg245His)	˃10 adenomas (44)	right-sided (43)	bifocal breast cancer (44)	MGF:rectal (65)	/
2.	c.933+3A>C p? c.933+3A>C p?	˃50 adenomas + sessile serrated polyps (63)	/	endometrial cancer (55)	S:ovarian (36) M: breast (82)	/
3.	c.734G>A p. (Arg245His) c.1147delC p. (Ala385Profs*23)	10 adenomas (43)	right-sided (43)	ovarian mucinous cystadenoma (43)	PU:larynx (61)	/
4.	c.933+3A>C p? c.1187G>A p. (Gly396Asp)	˃50 adenomas (39)	/	breast cancer (49)*, thyroid nodules, hyperplastic gastric polyps	F:larynx (77) PA: breast (42) PA: lung (53) PGF: gastric (68)	/

*also carrier of heterozygous PV CHEK2:c.444+1G>A p?

MGF: maternal grandfather; S: sister; M: mother; PU: paternal uncle; F: father; PA: paternal aunt; PGF: paternal grandfather.

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P12.141.A MYH associated polyposis with a germline homozygous biallelic duplication in a Libyan pedigree

Nouha Bouayed ABDELMOULA, *Balkiss Abdelmoula, Samir Aloulou, Walid Smaoui, Kays Chaker, UR17ES36 Team*

Genomics of signalopathies at the service of medicine UR17ES36, Medical University, Sfax, Tunisia.

Objective: Colorectal adenomatous polyposis inherited in a recessive manner is associated to biallelic mutations in the MYH. Described in 2002, the recessively inherited MYH-associated polyposis (MAP) is a less severe variant of polyposis compared with familial adenomatous polyposis (FAP). Here, we describe a MAP Libyan family in who multiple cases were identified as having digestive cancers. Clinical observation: A 38-year-old Libyan patient was referred to our genetic counseling because of a colorectal cancer. Sanger sequencing was used for screening of APC and MYH mutations. The patient was born from a faraway consanguineous couple and reported the death of his sister, his maternal aunt and uncle at 45 year-old from digestive cancers. He was admitted with an acute digestive obstruction and during laparoscopy, a right hemicolectomy was conducted. A moderately differentiated Lieberkühnian adenocarcinoma arising in the ileocecal Bauhin's valve was removed. The tumor was associated to two other adenocarcinomas of the right colon. One of them arised from precursor adenomatous polyp. There was in fact multiple sessile polyposis. There was an infiltration of all the tunics of the colonic wall and part of the mesocolon with a vascular invasion and five lymph node metastasis with capsular rupture in two ganglions. At the molecular level, the patient has a homozygous biallelic MYH mutation at the exon 13 of the gene: the MUTYH c.1185_1186dupGG variant resulting in a premature termination of the protein.

Conclusion: The germline homozygous duplication c.1185_1186dup is one of the most frequent genetic alteration in North African families with MAP.

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P12.142.B Next generation sequencing for germline mutation analysis in patients with neurofibromatosis

***Daniela Pencheva**, Kunka Kamenarova, Kalina Mihova, Ivanka Dimova, Vanio Mitev, Radka Kaneva*

Molecular medicine center, MU- Sofia, Sofia, Bulgaria.

Introduction: Neurofibromatosis of types 1 and 2 (NF1, NF2) and schwannomatosis form part of rare tumor-suppressor syndromes called neurofibromatosis. The neurofibromatosis give rise to a greater tumor burden for the nervous system than any other neoplastic disease.

Materials and Methods: In the current study we included three Bulgarian patients diagnosed with NF1 and two patients diagnosed with NF2. DNA was isolated from blood. Next-generation sequencing was performed on MiSeq/Illumina platform with a panel of 94 cancer related genes.

Results: We found three heterozygous pathogenic variants in *NF1* gene, encoding the tumor suppressor protein neurofibromin, and one heterozygous pathogenic variant in *NF2* gene encoding the tumor suppressor protein merlin.

Conclusions: The missense variant c.2819C>G (p.Thr940Ser) in exon 21 of *NF1* gene has a very low population frequency ($1.655x10^{-5}$) in ExAC database. The other *NF1* mutation c.5839C>T (p.Arg1947Ter) in exon 39 is a pathogenic loss-of-function mutation. We found also a pathogenic deep intronic variant in *NF1* gene c.4110+945A>G. These type of mutations form new or enhance the effect of acceptor or donor splice sites, leading to the inclusion of non-coding exons in the template RNA and affecting protein function. The frequency of these variants is about 2% of all generating mutations in the *NF1* gene. In addition we found one mutation c.1737G>T (p.Lys579Asn) in exon 15 of *NF2* gene. This variant is new for data bases and affects the last base of exon 15, which according to the dbscSNV Splice Altering Predictions program is pathogenic. Funding: NSP "Young Scientists and Postdoctoral Fellows"

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P12.143.C Usefulness of a targeted NGS approach to prostate cancer: two case reports of homologous recombination repair genes involvement

***Elisa De Paolis**, Paola Concolino, Andrea Urbani, Angelo Minucci*

Fondazione Policlinico Universitario A. Gemelli IRCCS, 00168, Italy.

Introduction: The findings about the role of Homologous Recombination Repair (HRR) genes in Prostate Cancer (PC) risk, lead to the inclusion of a target genetic testing into the last clinical practice guidelines. Moreover, an approved molecular targeted therapy is now available. Here we described two cases of PC harboring HRR mutations detected through a Next Generation Sequencing (NGS) multi-genes panel.

Materials and Methods: Case 1 is a young man with localized PC and family history of PC. Case 2 is a man with aggressive PC and a family history of Breast Cancer. The molecular evaluation was firstly performed on tissue samples using the 14-genes NGS panel HBOC (Devyser). Successively, a germline test was assessed.

Results: From tissue sample evaluation of case 1, we observed the co-occurrence of two different pathogenic variants (one of germline origin) and a somatic Copy Number Variants in *BRCA2* gene. The evaluation of case 2 described a germline CNV involving *ATM* gene.

Conclusions: We reported two PC affected subjects tested as positive for *BRCA2* and *ATM* genes alterations. Among the genes with a role in HRR, *BRCA2* and *ATM* are the most commonly reported. Information regarding HRR genes status in patients with PC is useful to target treatments (PARPi) or to the eligibility for clinical trials. However, comparing to other malignancies, only a minority of PC patients underwent a molecular analysis. Given its novelty, we want to support the relevance of HRR molecular profiling in PC affected subject.

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P12.144.D Medulloblastoma in a patient with a balanced t (5;22)(q35.1;q11.2) affecting the NF2 gene

***Lucia Roque**[1], Claudia Faria[2], Sofia Nunes[3], José Pimentel[4]*

[1]Molecular and Pathology Investigation Unit (UIPM), Portuguese Cancer Institute, Lisbon, Portugal, [2]Neurosurgery Department, Centro Hospitalar Lisboa Norte, Lisbon, Portugal, [3]Pediatric Neurology Department, Portuguese Cancer Institute, Lisbon, Portugal, [4]Neuropathology Department, Centro Hospitalar Lisboa Norte, Lisbon, Portugal.

Introduction: Neurofibromatosis type 2 (NF2) is an autosomal dominant syndrome caused by inactivating alterations in the NF2 gene on chromosome 22q11.2. NF2 characteristically predisposes to the development of vestibular schwannomas, meningiomas, ependymomas and gliomas.

Material and Methods: We report the case of a 10-year-old girl with a clinical history of NF2 that was submitted to surgical resection of a posterior fossa tumor in the cerebellum, and diagnosed as a medulloblastoma NOS with leptomeningial dissemination. Genetic analysis of the tumor biopsy was performed by conventional cytogenetic analysis, HR-CGH and FISH. The constitutional karyotype of the patient and parents was also evaluated.

Results: Cytogenetic analysis of the tumor cells revealed the following karyotype: 46,XX, t(5;22)(q35.1; q11.2), der(22) t(5;22)(q35.1;q11.2)[21]. CGH showed an unbalanced genome with multiple gains and losses namely, loss of 22q11.2-q13. The patient constitutional karyotype was: 46, XX; t(5;22)(q35.1;q11.2). The parent's karyotypes were normal. FISH analysis with a NF2 probe revealed that this gene was rearranged. In tumor cells, loss of the two copies of NF2-3' green probe occurred. No losses were observed in the patient blood lymphocytes.

Conclusions: To our knowledge, this is the second report of a medulloblastoma in a patient with NF2 and the first where genetic analysis was performed. Due to the rarity of the association between NF2 and medulloblastoma we hypothesize that, the rearrangement of a gene at chromosome 5q35.1, in the context of other abnormalities present in cerebellar cells,promoted the development of medulloblastoma.Grant: UIC1230-IPOFG

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P12.145.A The influence of non-coding RNAs on the genes of epigenetic regulation in gastric cancer

***Irina V. Bure**[1], Ekaterina A. Vetchinkina[1], Alexey I. Kalinkin[2], Ekaterina B. Kuznetsova[1], Marina V. Nemtsova[1]*

[1]Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russian Federation, [2]Research Center for Medical Genetic, Moscow, Russian Federation.

Introduction: Deregulation of epigenetic mechanisms and non-coding RNA (ncRNA) expression play a significant role in tumorigenesis. That make them prominent clinical markers to predict a prognosis and choose the appropriate therapy. Interactions between ncRNAs and methylation were demonstrated. The aim of our study was to investigate correlations of expression of ncRNAs and genes of epigenetic regulation in gastric cancer.

Materials and methods: Total RNA was extracted from normal and tumor tissue of gastric cancer patients using Trizol. Following cDNA synthesis, the expression of mRNA, miRNA, and lncRNAs in samples was analysed by using MiScript SYBR Green PCR Kit and QuantiTect SYBR Green PCR Kit (Qiagen). The housekeeping genes LMNB1, RNU6B and HPRT1 were used as controls.

Results: The investigation of 25 genes of epigenetic regulation using the custom targeted NGS-panel and genomic databases revealed functional transcripts in their introns, including both known (linc00847 (EZH2), SIRLNT (SIRT1), TERT (SMARCA4), PROX1-AS1 (PROX1)) and previously undescribed lncRNAs (HDAC2-AS2 and LOC101929089). The analysis of previous results by mirDIP algorithm determined miRNAs related to epigenetic regulation, and the most prominent candidate were also included in the study, namely miR-1301-3p, miR-106a-5p, miR-129-5p, miR-3613-3p, miR-548c-3p, miR-647. The expression of the transcripts in gastric cancer samples was determined and further analysis of their correlations currently in process.

Conclusions: Comparison of aberrant expression of ncRNAs participating in epigenetic regulation with clinical characteristics will allow to evaluate their diagnostic and prognostic potential in gastric cancer. The authors received funding from the Russian Science Foundation, Ref. No. 20-75-10117, for this work.

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P12.146.B Familial non-medullary thyroid cancer: clinical features and molecular analysis

Claire Forde[1], *John Fussey*[2], *Claire Hartley*[1], *George Burghel*[1], *John Hoffman*[3], *Julian Adlard*[4], *Fiona Lalloo*[1], *Lucy Side*[5], *Patrick J. Morrison*[6], *Joel Smith*[7], *Sara Dyer*[3], *Inigo Ortiz de Mendibil Ayuso*[3], *Derek Lim*[3], *Dafydd G. R. Evans*[1], *Eamonn R. Maher*[8], *Emma R. Woodward*[1]

[1]Manchester Centre for Genomic Medicine, Manchester Universities NHS Foundation Trust, Manchester, United Kingdom, [2]Department of Otolaryngology, University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom, [3]West Midlands Regional Genetics Service, Birmingham Women's NHS Foundation Trust, Birmingham, United Kingdom, [4]Yorkshire Regional Genetics Service, Leeds Teaching Hospitals NHS Trust, Leeds, United Kingdom, [5]Wessex Clinical Genetics Service, University Hospital Southampton NHS Foundation Trust, Southampton, United Kingdom, [6]Northern Ireland Regional Genetics Service, Belfast Health and Social Care Trust, Belfast, United Kingdom, [7]Peninsula Clinical Genetics Service, Royal Devon and Exeter Foundation Trust, Exeter, United Kingdom, [8]East Anglia Regional Genetics Service, Cambridge University Hospitals, Cambridge, United Kingdom.

Introduction: Familial non-syndromic medullary thyroid cancer (FNMTC) accounts for up to 5% of NMTC. Although germline alterations in candidate genes e.g. SRGAP1, SRRM2, FOXE1; have been identified in FNMTC, these have been in isolated individuals or single/paired families.

Materials and Methods: A clinical investigation was undertaken in 16 kindreds containing two or more cases of NMTC. Syndromic cases were excluded. To identify possible predisposing genetic factors, germline exome sequencing was completed in 4 kindreds (8 individuals), along with somatic copy number variant (CNV) and loss of heterozygosity (LoH) analyses in Formalin-Fixed Paraffin-Embedded tissue.

Results: Thirty-five affected individuals (9 male, 26 female) were included. There was a 2.9-fold excess of females [26/35(74%)], similar to the ratio in published cohorts of sporadic cases ($P = 0.8514$). Mean age of diagnosis was 41.6 years, significantly younger than sporadic cases (mean = 46.9y, $P = 0.0389$). Fourteen patients had multifocal and 13 had unifocal disease (8 unknown) and 7/25 (28%) had extra-thyroid extension disease. Exome sequencing did not detect any (likely) pathogenic variants in the known NMTC predisposition genes. Novel candidates discrete to individual kindreds included a frameshift variant in CNTNAP3 (tumour CNV loss in both affected family members), a missense variant in LMNB2 (tumour CNV loss concordant in 1/3 siblings), and a missense variant in GAPVD1 (CNV loss and LoH in both affected family members).

Conclusions: Epidemiological evidence suggests an inherited predisposition to FNMTC (young age at diagnosis, multifocal disease). A number of candidate genes have been identified; however further susceptibility loci are likely to contribute to familial cases.

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P12.147.C Frequency of heterozygous carriage of mutations in the NOTCH signaling pathway genes in clear cell renal cell carcinoma patients & in populations of the Volga-Ural region

Elizaveta Ivanova[1], *Irina Gilyazova*[1], *Galiya Gimalova*[1], *Adel Izmailov*[2], *Ilnur Sultanov*[2], *Gulshat Gilyazova*[2], *Valentin Pavlov*[2], *Elza Khusnutdinova*[1]

[1]Institute of Biochemistry and Genetics - Subdivision of the Ufa Federal Research Centre of the Russian Academy of Sciences, Ufa, Russian Federation, [2]Bashkir State Medical University, Ufa, Russian Federation.

Introduction: Transmembrane receptors of the Notch family carry out regulatory actions, affecting proliferation, apoptosis, differentiation, angiogenesis, metastasis, and other cellular processes that induce the onset and development of malignant tumors including renal cell carcinoma. We aimed to determine the frequency of mutations in the Notch signaling pathway (*DLL4* (rs35748882), *HEY2* (rs61737181), *JAG1* (rs1801140, rs1801139, rs45575136), *NOTCH1* (rs61751542), *NOTCH2* (rs3795666), *NOTCH4* (rs8192576, rs8192579, rs8192585) identified earlier as a result of exome sequencing in an expanded group of patients with clear cell renal cell carcinoma.

Materials and Methods: The study included 238 paired samples of tumor and normal kidney tissue from patients with clear cell renal cell carcinoma and 150 samples of population controls. Detection of nucleotide sequence alterations of genes was performed using PCR followed by RFLP analysis. Restriction enzymes were selected using the NEBcutter V2.0 Internet resource.

Results: On average, the frequency of detected changes in the group of clear cell renal cell carcinoma patients was higher than the general population values. The highest frequency was found for rs8192579 and rs8192585 *NOTCH4* gene. Clinical and pathological characteristics of the tumors in which mutations were identified, were heterogeneous and included patients with both early and late stages.

Conclusions: The results obtained in this study may indicate the contribution Notch signaling pathway gene alterations to the pathogenesis of clear cell renal cell carcinoma, as well as the possibility of their use in creating a molecular markers panel for the diagnosis and prognosis of the course of the disease.

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P12.148.D Association of novel germline MLH1 in-frame deletion with uncommon isolated PMS2 loss in tumor tissue

Gasper Klancar[1], *Ana Blatnik*[2], *Vita Setrajcic Dragos*[1], *Vesna Vogric*[1], *Vida Stegel*[1], *Olga Blatnik*[3], *Primož Drev*[3], *Barbara Gazic*[3], *Mateja Krajc*[2], *Srdjan Novakovic*[1]

[1]*Institute of Oncology, Division of Diagnostics, Department of Molecular Diagnostics, SI-1000 Ljubljana, Slovenia,* [2]*Institute of Oncology Ljubljana, Cancer Genetics Clinic, Ljubljana, Slovenia, SI-1000 Ljubljana, Slovenia,* [3]*Institute of Oncology Ljubljana, Department of Pathology, Institute of Oncology Ljubljana, SI-1000 Ljubljana, Slovenia.*

Introduction: Lynch syndrome (LS) diagnostics is based on the detection of DNA-mismatch-repair (MMR) system deficiency. MMR-deficiency can be detected in tumor tissue by microsatellite instability (MSI) using molecular test or by loss of expression of MMR proteins using immunohistochemistry (IHC). According to NCCN guidelines, definitive LS diagnosis requires identification of germline pathogenic variant (PV) in one of the MMR genes.

Materials and Methods: According to the sample availability, we performed testing of the tumor-FFPE and blood/non-tumor-FFPE samples in one LS suspected family. All FFPE samples were IHC stained for MMR proteins and subsequently tested for MSI using in-house-method. Selected tumor and blood samples were tested for genetic alterations with NGS sequencing using TruSight_Cancer_Panel-(blood) or TruSight_Tumor_170-(tumor). Segregation analysis and confirmation of NGS data was performed with Sanger sequencing. Variants were classified according to ACMG_guidelines.

Results: We discovered novel *MLH1* in-frame-deletion LRG_216t1:c.2236_2247delCTGCCTGATCTA p.(Leu746_Leu749del) associated with LS. Variant appears to be associated with uncommon isolated loss of PMS2-protein in tumor tissue instead of MLH1 and PMS2 protein loss, which is commonly seen with PVs in MLH1. The variant was classified as likely pathogenic, based on segregation analysis and molecular characterization of tumor and blood samples.

Conclusions: Proven variant co-segregation with affected family members emphasizes the importance of linking clinical and molecular data. A functional study will be needed to conclusively determine effect of variant on the interaction of MLH1 and PMS2. This report contributes to characterization of PV spectra in MLH1 leading to LS.

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P12.149.A *NTHL1*-tumor syndrome in Slovenian patients with adenomatous polyposis

Iva Opalic[1], *Ksenija Strojnik*[2], *Mateja Krajc*[2], *Marta Banjac*[2], *Vida Stegel*[3], *Petra Skerl*[3], *Vita Setrajcic Dragos*[3], *Gasper Klancar*[3], *Srdjan Novakovic*[3], *Ana Blatnik*[2]

[1]*University Medical Centre Maribor, Maribor, Slovenia,* [2]*Cancer Genetics Clinic, Institute of Oncology Ljubljana, Ljubljana, Slovenia,* [3]*Department of Molecular Diagnostics, Institute of Oncology Ljubljana, Ljubljana, Slovenia.*

Introduction: *NTHL1*-associated polyposis or *NTHL1*-tumour syndrome is an autosomal recessive cancer predisposition. *NTHL1* deficiency predisposes to adenomatous polyposis and colorectal cancer, but appears to be associated with moderate to high risk of breast cancer and other tumour types as well.

Materials and methods: We performed a retrospective analysis of our adenomatous polyposis patient cohort to identify those with biallelic *NTHL1* pathogenic variants (PVs). We only included patients who had ≥10 histologically confirmed tubular adenomas and were tested with NGS panels for germline variants in the years 2015-2020.

Results: Out of 72 patients with adenomatous polyposis, 2 (2.8%) had biallelic PVs in *NTHL1*. Patient 1 was a female, aged 62, who had >10 adenomatous polyps removed on each yearly colonoscopy. She also had endometrial cancer at age 45, colon cancer at age 48, breast cancer at age 55, and diffuse gastric cancer at age 62. Three of her siblings also had breast, colon and gastric cancer respectively. She was a carrier of a homozygous PV in *NTHL1*, c.268C>T p.(Gln90*). Patient 2 was also a female, aged 79, who had more than 50 colorectal adenomas removed on three colonoscopies in less than 2 years. She was also diagnosed with breast cancer at age 47 and with non-Hodgkin lymphoma at age 74. Her family history was positive for colon cancer in two first-degree relatives. She carried two *NTHL1* PVs, c.268C>T p.(Gln90*) and c.235dupG p.(Ala79Glyfs*2).

Conclusion: Both of our patients with *NTHL1* deficiency and adenomatous polyposis had various extracolonic disease manifestations, including breast cancer.

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P12.151.C Germline mutations in *TP53* and *BRCA1* genes in pediatric patients with osteosarcoma revealed by multigene panel testing

Vera V. Semenova[1,2,3], *Valentina M. Kozlova*[2], *Elena V. Zhukovskaya*[3], *Svetlana N. Mikhailova*[2], *Alexandr F. Karelin*[3], *Tatiana V. Nasedkina*[1,3]

[1]*Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russian Federation,* [2]*N.N. Blokhin National Medical Research Center of Oncology, Moscow, Russian Federation,* [3]*Dmitry Rogachev National Medical and Research Center of Pediatric Hematology, Oncology and Immunology, Moscow, Russian Federation.*

Introduction: Osteosarcoma is the most common bone tumor in children and young adults. A significant proportion of osteosarcomas is associated with germline mutations in cancer predisposition genes.

Patients and Methods: A total of 18 patients with osteosarcoma were enrolled in the study. There were 9 girls and 9 boys aged 3-17 years (mean age 12,1). Six patients had multiple primary tumors. In addition to osteosarcoma they had nephroblastoma (n = 2), soft tissue sarcoma (n = 3), retinoblastoma (n = 1). Genomic DNA was isolated from peripheral blood leukocytes and next-generation sequencing (NGS) on the NextSeq500 Illumina platform was performed. A multigene panel included coding sequences of 882 cancer-associated genes. The library was prepared using enrichment by hybridizationwith NimbleGen probes (Roche).

Results: Mutations in *TP53* gene were revealed in four patients (4/18). Among them, two siblings with osteosarcoma carried pathogenic mutation.524G>A (rs28934578), their mother had breast cancer at age 35. A girl with osteosarcoma and nephroblastoma had variant with uncertain clinical significance c.631A>C (rs1060501198). Her father with renal cell carcinoma also was the mutation carrier. A patient with osteosarcoma and embryonic rhabdomyosarcoma without family history had pathogenic mutation c.725G>A (rs121912655). Another affected gene found in our patients was *BRCA1*. A girl with osteosarcoma carried *BRCA1* 2080delA mutation, her mother and grandfather with Hodgkin's lymphoma and gastric cancer were heterozygous carriers.

Conclusions: Germline mutations in cancer associated genes were found in 27% of our patients. Most frequent were mutations in *TP53* genes (4/5), that means that in 22% patients osteosarcoma was a part of Li-Fraumeni syndrome.

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P12.152.D Analysis of *BARD1, PRDM9, RCC1,* and *RECQL* in patients with ovarian cancer by targeted next-generation sequencing of DNA pools

Malwina Suszynska[1], *Magdalena Ratajska*[2,3], *Aleksandra Ryszkowska*[1], *Jaroslaw Debniak*[4], *Dariusz Wydra*[4], *Cezary Cybulski*[5], *Bartosz Wasag*[2,6], *Piotr Kozlowski*[1]

[1]*Department of Molecular Genetics, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland,* [2]*Department of Biology and Medical Genetics, Medical University of Gdansk, Gdansk, Poland,* [3]*Department of Pathology, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand,* [4]*Department of Gynaecology, Oncologic Gynaecology and Gynaecological Endocrinology, Medical University of Gdansk, Gdansk, Poland,* [5]*Department of Genetics and Pathology, International Hereditary Cancer Center, Pomeranian Medical University, Szczecin, Poland,* [6]*Laboratory of Clinical Genetics, University Clinical Centre, Gdansk, Poland.*

Introduction: Several ovarian cancer susceptibility genes have been discovered, but more are likely to exist. In this study, we aimed to analyze knowledge-based selected candidate susceptibility genes, i.e., *BARD1*, *PRDM9*, *RCC1*, and *RECQL*, in which loss-of-function germline mutations have been reported in patients with breast and/or ovarian cancer.

Materials and Methods: We analyzed the exons of *BARD1*, *PRDM9*, *RCC1*, and *RECQL* (plus 3'- and 5'- 25 flanking bases) of ~400 patients with ovarian cancer by targeted next-generation sequencing of 25 pooled DNA samples (16 individuals/pool, including several positive-control and duplicated samples). SureCall application (Agilent) was used for end-to-end data analysis. The identified rare genetics variants were validated by Sanger sequencing in all individual samples of the pool carrying the variant.

Results: No pathogenic mutation was found in the targeted candidate susceptibility genes. Validation genotyping of filtered rare silent and missense variants revealed that the majority of them were true variants. In addition, the high concordance (R^2=0.95) of population allele frequency for 43 common SNPs in the control European population (gnomAD) and our experiment confirmed the reliability of pooled sequencing.

Conclusions: Mutations in *BARD1*, *PRDM9*, *RCC1*, and *RECQL* do not contribute substantially to the risk of ovarian cancer. Pooled DNA sequencing is a cost-effective and reliable method for the initial screening of candidate genes.

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P12.153.A Use of ovarian cancer tumour pathology characteristics to aid classification of Variants of Uncertain Significance (VUS) in the *BRCA1* and *BRCA2* genes

Denise O' Mahony[1,2,3], *Susan J. Ramus*[4,5], *Melissa Southey*[6,7,8], *Andreas Hadjisavvas*[2,3], *Nicola S. Meagher*[4,5], *Antonis C. Antoniou*[9], *Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA), Ovarian Tumor Tissue Analysis Consortium (OTTA), David Goldgar*[10], *Amanda Spurdle*[11], *Kyriaki Michailidou*[1,3]

[1]*Biostatistics Unit, the Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus,* [2]*Department of Electron Microscopy and Molecular Pathology, the Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus,* [3]*Cyprus School of Molecular Medicine, Nicosia, Cyprus,* [4]*School of Women's and Children's Health, Faculty of Medicine, University of New South Wales, Sydney, Australia,* [5]*Adult Cancer Program, Lowy Cancer Research Centre, University of New South Wales, Sydney, Australia,* [6]*Monash University, Precision Medicine, School of Clinical Sciences at Monash Health, Clayton, Australia,* [7]*Department of Clinical Pathology, The University of Melbourne, Melbourne, Australia,* [8]*Cancer Epidemiology Division, Cancer Council Victoria, Melbourne, Australia,* [9]*Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, Cambridge, United Kingdom,* [10]*Huntsman Cancer Institute, University of Utah School of Medicine, Department of Dermatology, Salt Lake City, UT, USA,* [11]*Division of Genetics and Population Health, QIMR Berghofer Medical Research Institute, Brisbane, Australia.*

Introduction: Individuals carrying pathogenic/likely pathogenic variants in the *BRCA1/2* genes have a high lifetime risk of developing breast and ovarian cancer. Diagnostic DNA testing identifies carriers who could benefit from specialized care. The increase in the use of genetic testing and particularly multi-gene panel testing, has increased the number of variants of uncertain clinical significance (VUS) being detected, carriers of which do not benefit from life-saving interventions. The Multifactorial Likelihood Model evaluates VUS association with disease risk in hereditary cancer genes by integrating multiple lines of evidence. This model currently does not consider ovarian cancer characteristics. We aim to assess ovarian cancer characteristics as predictors of *BRCA1/2* variant pathogenicity, for inclusion in the Multifactorial model and as clinical data points using the American College of Medical Genetics and Genomics and Association for Molecular Pathology (ACMG/AMP) quantitative evidence system.

Methods: Histopathology/morphology tumour information was obtained from ovarian cancers, in *BRCA1/2* pathogenic variant carriers (n = 1,942) and *BRCA1/2* non-carriers (n = 2,616). Likelihood Ratios (LR) were calculated for each histopathological feature, and were aligned to ACMG/AMP strengths.

Results: 'High-grade serous' tumours are positive predictors of pathogenicity for the *BRCA1/2* genes (LRs = 1.15-1.16), while 'Other' tumours are positive predictors for *BRCA1* pathogenicity (LR = 1.46). 'Mucinous' (LR = 0.17-0.30) and 'Clear-cell' (LR = 0.22-0.16) tumours negatively predict *BRCA1/2* pathogenicity and reach 'Supporting' to 'Moderate' benign evidence under ACMG/AMP rules. The above histopathological features reach significance and can be incorporated in future multifactorial models.

Conclusions: We provide refined estimates for predicting *BRCA1/2* variant pathogenicity using ovarian tumour characteristics that will significantly improve variant interpretation.

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P12.154.B Using FFPE tissue from non-mucinous epithelial ovarian cancer patients to detect germline variants

José Luis Villanueva-Cañas[1], *Míriam Potrony*[2,3], *Adela Saco*[4], *Ricard Isanta*[1], *Vanesa Lopez*[1], *Victor Pastor*[1], *Elena Gonzalvo Mallón*[4], *Mireia García Gerona*[4], *Lorena Moreno*[5], *Belén Pastor*[6], *Eduardo González*[7], *Bárbara Adamo*[5], *Lydia Gaba*[8], *Aurora Sánchez*[2], *Pedro Jares*[1,4], *Joan Antón Puig-Butillé*[1,2]

[1]*Molecular Biology CORE Laboratory, Hospital Clínic de Barcelona, Barcelona, Spain,* [2]*Biochemical and Molecular Genetics Department, Hospital Clínic de Barcelona, Barcelona, Spain,* [3]*Centro de Investigación Biomédica en Red en Enfermedades Raras, Instituto de Salud Carlos III, Barcelona, Spain,* [4]*Pathology Department, Hospital Clínic de Barcelona, Barcelona, Spain,* [5]*Gastroenterology Department, Hospital Clínic de Barcelona, Barcelona, Spain,* [6]*Medical Oncology, Hospital Clínic de Barcelona, Barcelona, Spain,* [7]*Obstetrics and Gynecology Department, Hospital Clínic de Barcelona, Barcelona, Spain,* [8]*Medical Oncology Department, Hospital Clínic de Barcelona, Barcelona, Spain.*

Introduction: Genetic testing of homologous recombination DNA repair pathway related genes is relevant in ovarian cancer patients. This genetic testing allows for cancer risk identification and guides first-line treatment selection since PARP1 inhibitors are approved for germline and/or somatic BRCA-mutated advanced ovarian cancer. This study assess the feasibility of DNA analysis from FFPE tissue as a resource for initial genetic testing in ovarian cancer patients.

Materials and Methods: We selected twenty-five non-mucinous epithelial ovarian cancer patients based on their germline genetic background, corresponding to six patients with germline *BRCA1/2* mutations, six patients with germline mutations in *ATM, BRIP1, CHEK2* or *PALB2* genes, and thirteen wild-type patients. In all patients, somatic DNA from the FFPE ovarian tumour was analyzed by the Oncomine BRCA Expanded NGS panel (ThermoFisher) and a commercial bioinformatic pipeline. The analysis was focused on *BRCA1, BRCA2, ATM, CHEK2, BRIP1, BARD1, PALB2, ATM*, and *TP53* genes.

Results: The sensitivity of detecting a germline mutation in FFPE DNA tissue was 92%. Among wild-type germline patients, a somatic mutation in *ATM* and *BRCA1/2* and was observed in two and three patients, respectively. All somatic mutant *BRCA1/2* tumours were classified as high-grade serous carcinomas. In addition, somatic TP53 mutations were observed in 73% of tumours.

Conclusions: An initial genetic testing using routine FFPE tissue is efficient for the detection of germline and somatic mutations in *BRCA1/2* and other genes involved in homologous recombination DNA repair pathway, including several INDELs.

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P12.155.C Weighted gene co-expression network analysis of ovarian cancer transcriptional profile and its relations to stemness

Anna Erol[1], *Magdalena Niemira*[1], *Karolina Chwiałkowska*[2], *Anna Szałkowska*[1], *Agnieszka Bielska*[1], *Justyna Raczkowska*[1], *Gabriela Sokołowska*[1], *Iwona Sidorkiewicz*[1], *Katarzyna Doroszko*[1], *Katarzyna Doroszko*[1], *Patrycja Modzelewska*[3], *Mirosław Kwaśniewski*[2], *Joanna Reszeć*[3], *Jacek Szamatowicz*[4], *Paweł Knapp*[5], *Marcin Moniuszko*[6], *Adam Krętowski*[1,7]

[1]*Clinical Research Centre, Medical University of Bialystok, Bialystok, Poland,* [2]*Centre for Bioinformatics and Data Analysis, Medical University of Bialystok, Bialystok, Poland,* [3]*Departament of Medical Pathomorphology, Medical University of Bialystok, Bialystok, Poland,* [4]*Department of Gynecology and Gynecological Oncology, Medical University of Bialystok, Bialystok, Poland,* [5]*University Oncology Centre, Medical University of Bialystok, Bialystok, Poland,* [6]*Department of Regenerative Medicine and Immune Regulation, Medical University of Bialystok, Bialystok, Poland,* [7]*7Department of Endocrinology, Diabetology and Internal Medicine, Medical University of Bialystok, Bialystok, Poland.*

Introduction: Unsupervised approaches like Weighted Gene Co-expression Network Analysis (WGCNA), allow data reduction of high dimensional sequencing data, by creating modules, based on pairwise correlation. These modules can be further related to additional sample information. Cancer stem-like cells (CSC) hypothesis, gains researchers' interest as evidence are rising. The hypothesis states, that cells subpopulation showing stemness characteristics or are at stemness state are promoting tumour development, relapse and metastasis. The goal of this study is to find modules of genes related to CSC.

Methods: The WGCNA has been performed on paired-end total RNA sequencing of 33 Non-Tumour and 33 High-Grade Serous Ovarian Cancer on-site generated data. Modules have been correlated with mRNAsi and other clinical information available for patients. Downstream analysis of chosen modules followed.

Results/Conclusions: Two modules have been significantly correlated with mRNAsi: module yellow and turquoise Gene ontology (GO) enriched in the yellow module include nuclear division and top enriched KEGG pathway is cell cycle. GO for module turquoise top enriched biological processes are mostly related to development and KEGG enrichment top results include Wnt, Hippo, TGF-beta signalling pathways. These results will be further validated by sequencing of CSC-enriched subpopulation of ovarian cancer cells. This research was conducted within the project which has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 754432 and the Polish Ministry of Science and Higher Education, from financial resources for science in 2018-2023 granted for the implementation of an international co-financed project.

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P12.156.D Identification of novel ovarian cancer susceptibility genes through investigation of exceptional responders to platinum-based therapy

Anna Sokolenko[1,2], *Valeria Ni*[1], *Tatiana Sokolova*[1], *Robert Broyde*[3], *Thilo Dörk*[4], *Evgeny Imyanitov*[1,2]

[1]*N.N. Petrov Institute of Oncology, St-Petersburg, Russian Federation,* [2]*St. Petersburg Pediatric Medical University, St.-Petersburg, Russian Federation,* [3]*City Cancer Center, St-Petersburg, Russian Federation,* [4]*Hannover Medical School, Hannover, Germany.*

Introduction: A significant share of ovarian cancer (OC) incidence is attributed to inherited germline mutations. Virtually all known genes for hereditary OC, e.g., *BRCA1*, *BRCA2*, *RAD51C*, *RAD51D*, render pronounced tumor sensitivity to both platinum compounds and PARP inhibitors. Here, we present an approach to identifying new genetic determinants of OC predisposition by analyzing exceptional responders to platinum-based therapy.

Material and method: 117 patients diagnosed with advanced high-grade serous ovarian cancer were selected for the study based on the criteria of the exceptional response to treatment, i.e. the complete clinical or pathological response to chemotherapy coupled with the disease-free interval of at least 12 months. Genomic DNA was sequentially subjected to *BRCA1/BRCA2* analysis, followed by 25-gene panel targeted sequencing of *BRCA1/BRCA2* mutation-negative cases and whole-exome sequencing.

Results: A significant enrichment for *BRCA1/BRCA2* germline mutations was observed: 37/117 (32%) analyzed patients carried pathogenic *BRCA1* or *BRCA2* alleles. Upon targeted sequencing, we identified 6 carriers of pathogenic alleles in *NBN* (n = 3), *ATM* (n = 1), *PALB2* (n = 1), and *RAD51D* (n = 1) genes. The analysis of 40 *BRCA1/BRCA2*-negative cases by whole-exome sequencing revealed several protein-truncating variants in genes involved in DNA repair or bearing tumor suppressor function: *BRIP1*, *FANCM*, *RAD50*, *RAD54B*, *STK36*, *POLA2*, *PTCH2*, *AEN*, *MSH4*.

Conclusion: Irrespective of family history, exceptional responders to platinum-based therapy represent a genetically-enriched cohort of patients eligible for identifying novel genes for hereditary OC. This work is supported by the Russian Foundation for Basic Research [grant number 20-515-12009].

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P12.157.A Proposal of new candidate genes of predisposition to serous ovarian cancer using whole-exome-sequencing of 16 patients with a familial form

Mathias Cavaillé[1], ***Maud Privat***[1], *Lorenzo Menicucci*[1], *Flora Ponelle-Chachuat*[1], *Nicolas Sonnier*[1], *Sandrine Viala*[1], *Mathis Lepage*[1], *Mathilde Gay-Bellile*[1], *Nancy Uhrhammer*[1], *Yannick Bidet*[2], *Yves-Jean Bignon*[1]

[1]*Centre Jean Perrin, Clermont Ferrand, France,* [2]*Université Clermont Auvergne, Clermont Ferrand, France.*

High-grade serous ovarian cancer is associated with a hereditary predisposition to cancer identified in almost 1/4 of cases. However, no causal pathogenic variant is found in more than 50% of familial forms, suggesting the existence of other risk factors, including other high risk genetic factors. Exploration of 16 patients presenting a familial form of high grade serous ovarian carcinoma using whole-exome sequencing was performed in our study. The rare variants shared by at least 2 unrelated patients were selected, including truncating, splicing and missense variants from a list of known or suspected genes involved in oncogenesis. Their interpretation using different databases found twelve candidate genes and three candidate pathways, including Hippo-YAP/TAZ pathways. The missense variants were then studied in tumor samples, looking for loss of heterozygoty and/or loss of expression. Functional in vitro studies are also currently being carried out to clarify the involvement of these new genes in ovarian carcinogenesis. In conclusion, our study design using exome analysis of patients with a familial form of ovarian cancer identified candidate genes for hereditary predisposition to high-grade serous ovarian cancer. Further investigations, including a large case - control study, are required to confirm their implication in predisposition to ovarian carcinoma.

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P12.160.D Investigation of Wnt signaling pathway components in VPA induced PANC 1 cells

Yeliz Ekici[1,2], *Abdullah Yilmaz*[3], *Umut Kucuksezer*[3], *Sema Bilgic Gazioglu*[3], *Zeynep Dogusan Yamalioglu*[4,5], *Ali Osman Gurol*[3,6], *Thomas Linn*[7], ***Feyza N. Tuncer***[1,6]

[1]*Istanbul University, Aziz Sancar Institute of Experimental Medicine Department of Genetics, Istanbul, Turkey,* [2]*Istanbul University, Graduate School of Health Sciences, Istanbul, Turkey,* [3]*Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Immunology, Istanbul, Turkey,* [4]*Yeni Yuzyil University Gaziosmanpasa Hospital Bone Marrow Transplantation Center, Cell Processing Unit, Istanbul, Turkey,* [5]*Yeni Yuzyil University, Medical Faculty, Department of Medical Microbiology, Istanbul, Turkey,* [6]*Istanbul University, Aziz Sancar Institute of Experimental Medicine, Diabetes Application and Research Center, Istanbul, Turkey,* [7]*7Justus-Liebig-University, Clinical Research Unit, Center of Internal Medicine, Giessen, Germany.*

Introduction: Pancreatic ductal adenocarcinoma (PDAC) is among the most aggressive cancers with a 5-year survival rate of less than 8%. Using PANC-1 cell line as a PDAC model, we aimed to examine the effect of valproic acid (VPA) on the expression of the canonical Wnt signaling pathway components and to determine IC_{50} of VPA with a novel approach.

Materials and Methods: PANC-1 cells were cultured for 24, 48, 72, or 96 h in the presence of VPA at 0.5, 1, 2.5, 5 and 10 mM/mL concentrations. Flow cytometry was utilized in assessing proliferative and apoptotic responses in cells by CFSE and Annexin V/PI staining, respectively. CFSE staining aided to calculate the difference between cell doublings via $2n = F_{control}/F_{VPA}$ through which graphs of VPA concentrations against durations were drawn to calculate the IC_{50} value. Taqman hydrolysis probes and SYBR Green PCR Mastermix were assessed in expression analyses of Wnt components utilizing $2^{-\Delta\Delta Ct}$ method.

Results: IC_{50} was calculated as 2.5 mM, at which cells were detected to undergo early apoptosis. At this concentration, a significant 4-fold upregulation in *LEF1* expression was detected.

Conclusions: We present a novel and specific calculation of IC_{50} value through combined analyses of CFSE and Annexin V/PI staining. Our approach enabled accurate and reproducible IC_{50} calculation at single cell level with no significant effect on the canonical Wnt signaling pathway. Further studies are needed to

clarify the role of *LEF1* in this model. This work was supported by Scientific Research Projects Coordination Unit of Istanbul University (TDP-2019-32763).

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P12.161.A The novel fusion transcripts in pediatric AML with complex 11q23 rearrangement

Lilit Ghukasyan[1], *Georgii Krasnov*[1], *Arina Bessonova*[1], *Liudmila Baidun*[2], *Tatiana Nasedkina*[1]

[1]*Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russian Federation,* [2]*Russian Children's Clinical Hospital, Moscow, Russian Federation.*

Acute myeloid leukemia (AML) with 11q23 rearrangement causing fusion of the *KMT2A* gene with various specific partner genes (AML-KMT2A-r), is one of the most common subtypes of pediatric AML (~18%). The clinical outcome depends mainly on partner gene and varies from intermediate to poor. About 100 gene-partners of *KMT2A* have been discovered so far, but many are still unknown. Thus, the detection of novel *KMT2A* chimeras in pediatric AML is important to improve the treatment stratification and disease monitoring. We performed RNA-seq of 9 samples obtained from 7 children with AML-KMT2A-r, a triplet of samples collected at diagnosis, remission and relapse was available for one patient. Only those AML-KMT2A-r cases were enrolled in the study in which FISH revealed atypical t(10;11) (n = 4) and in which FISH positive 11q23 rearrangement was not confirmed by reverse transcription-PCR (RT-PCR) (n = 3). All 4 patients with atypical t (10;11) showed complex rearrangements, carrying more than one fusion: patients 1, 2 and 7 harbored *MLLT10-DNAJC1*, *DNAJC1-KMT2A*; *FTH1-KMT2A*, *MLLT10-ANGPT1* and *KMT2A-MLLT10*, *MLLT10-*AP001107.9 fusions, respectively. Patient 4 with a triplet of samples carried *MLLT10-MKX, KMT2A-MLLT10* at diagnosis and relapse, no fusion in remission; also in relapse additionally emerged *TBL1XR1-EAF2* chimeric trancript was detected. In patients 5 and 6 with unconfirmed *KMT2A* fusions, RNA-seq revealed well known transcripts *KMT2A-SEPTIN5* and *KMT2A-MLLT11*, respectively. In patient 3 no rearrangement was found. Hence, we identified 5 novel fusions in pediatric leukemia patients: *DNAJC1-KMT2A, FTH1-KMT2A, MLLT10-ANGPT1, MLLT10-AP001107.9, MLLT10-MKX* and *TBL1XR1-EAF2*. The work was supported by Russian Science Foundation (grant # 18−15-00398).

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P12.162.B The management of Peutz-Jeghers syndrome: European Hereditary Tumour Group (EHTG) Guideline

Anja Wagner[1,2], *Stefan Aretz*[3,4], *Annika Auranen*[5], *Marco J. Bruno*[1,2], *Giulia M. Cavestro*[6], *Emma J. Crosbie*[7,8], *Anne Goverde*[1,2], *Anne Marie Jelsig*[9], *Andrew R. Latchford*[10,11], *Monique E. van Leerdam*[12,13], *Anna H. Lepisto*[14], *Marta Puzzono*[6], *Ingrid Winship*[15], *Veronica Zuber*[6], *Gabriela Möslein*[16]

[1]*University Medical Center Rotterdam, Rotterdam, Netherlands,* [2]*Erasmus MC Cancer Institute, Rotterdam, Netherlands,* [3]*University Hospital Bonn, Bonn, Germany,* [4]*University of Bonn, Bonn, Germany,* [5]*Tampere University Hospital, Tampere, Finland,* [6]*University IRCCS San Raffaele Scientific Institute, Milan, Italy,* [7]*Manchester Academic Health Science Centre, Manchester, United Kingdom,* [8]*, St Mary's Hospital, Manchester, United Kingdom,* [9]*University Hospital of Copenhagen, Copenhagen, Denmark,* [10]*Imperial College London, London, United Kingdom,* [11]*St. Marks Hospital, London, United Kingdom,* [12]*Netherlands Cancer Institute, Amsterdam, Netherlands,* [13]*Leiden University Medical Center, Leiden, Netherlands,* [14]*University Hospital of Helsinki, Helsinki, Finland,* [15]*University of Melbourne, Melbourne, Australia,* [16]*, Academic Hospital University of Düsseldorf, Duisburg, Germany.*

Introduction: Peutz-Jeghers syndrome (PJS) is a rare autosomal dominant condition characterized by mucocutaneous pigmentation and PJS hamartomatous polyps. In adulthood, PJS patients face an increased risk of different cancers. The scientific data to guide management of PJS are sparse. The European Hereditary Tumour Group (EHTG) commissioned an update of the previous guideline from 2010 (Beggs et al. Gut 2010).

Methods: Key clinical questions were identified and literature searches were performed using MEDLINE, Embase and Cochrane. The available evidence was reviewed and discussed. Evidence levels and recommendation strengths were assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE). A Delphi process was followed, with consensus being reached when ≥80% of the voting guideline committee members had voted either "Very strongly agree", "Strongly agree", or "Agree".

Results: On gastrointestinal and pancreatic management recent adequate guidelines were available. These were reviewed and endorsed after confirming no more recent relevant papers had been published. Literature searches were performed for additional questions and yielded a variable number of relevant papers depending on the addressed subject: 21/244 papers on genetic testing; 3/179 on gastrointestinal management; 6/177 on surgical management; 57/770 on pancreatic management; 16/302 on breast management and 6/188 on gynecological management were included. Additional recommendations and statements were formulated (Wagner et al. JCM 2021).

Conclusions: A decade later, the evidence base for recommendations remains poor and collaborative studies are required to provide better data in this rare condition. Within these restrictions, multisystem, clinical management recommendations for PJS have been formulated.

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P12.163.C Genetic study of a Tunisian family with Peutz-Jeghers syndrome

Marwa Mahdouani[1,2], *Mahdi Ksiaa*[3,4], *Badreddin Sriha*[5,4], *Rihen Brahem*[1], *Mohamed Denguezli*[6,4], *Moncef Mokni*[5,4], *Ali Saad*[1,4], *Moez Gribaa*[1,4]

[1]*Laboratory of Human Cytogenetics, Molecular Genetics and Reproductive Biology. Farhat Hached University Hospital, Sousse, Tunisia,* [2]*Higher Institute of Biotechnology of Monastir, Monastir, Tunisia,* [3]*Department of Gastroenterology. Sahloul University Hospital, Sousse, Tunisia,* [4]*Faculty of Medicine Ibn El Jazzar. University of Sousse, Sousse, Tunisia,* [5]*Department of Pathological Anatomy and Cytology. Farhat Hached University Hospital, Sousse, Tunisia,* [6]*Department of Dermatology and Venerology. Farhat Hached University Hospital, Sousse, Tunisia.*

Introduction: Peutz-Jeghers syndrome (PJS) is a rare inherited autosomal dominant disease, characterized by mucocutaneous perioral pigmentation, gastrointestinal hamartomatous polyps,

and an increased risk of malignancies such as colon, small intestine, stomach, breast, pancreas, lung, reproductive organs, and thyroid cancers. This disorder is caused by germline mutations in the tumor suppressor gene *STK11*, located on 19p13.3, encodes for the LKB1 protein comprising 433 amino acids and belonging to the serine/threonine kinase family. LKB1 regulates cellular responses involved in cell polarity, energy metabolism, cell growth, chromatin remodeling, angiogenesis, p53-dependent apoptosis, cell cycle arrest, fatty acid biosynthesis, and Wnt-signaling. Despite the high probability of a malignant transformation, molecular studies of PJS are rare in the world and particularly in Tunisia.

Materials and methods: Two PJS patients belonging to the same family (father and daughter) were sequenced for the open reading frame of *STK11* gene using the Sanger technique.

Results: We identified a novel frameshift variant; c.605-606insA (p.H202Qfs*265) at a heterozygous state in exon 5 of the *STK11* gene for the 2 patients.

Discussion: Most of the mutations associated with PJS are located in the kinase domain of the *STK11* gene, extending from exon 2 to exon 7, involved in substrate recognition. Several studies showed that an important proportion of mutations, including our variant, lead to a truncated protein and increases the risk of developing cancers.

Conclusion: Our results confirm that PJS is often associated with truncating mutations in the kinase domain of the *STK11* gene, which should be identified for an adequate genetic counseling.

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P12.164.D Yield of thyroid cancer surveillance in patients with *PTEN* Hamartoma Tumour Syndrome

Meggie M. C. M. Drissen[1], *Janet R. Vos*[1], *Martin Gotthardt*[2], *Nicoline Hoogerbrugge*[1]

[1]*Department of Human Genetics, Radboud university medical center, Nijmegen, Netherlands,* [2]*Department of Radiology and Nuclear Medicine, Radboud university medical center, Nijmegen, Netherlands.*

Introduction: Expert opinion-based guidelines recommend annual thyroid ultrasound surveillance (TUS) for patients with *PTEN* Hamartoma Tumour Syndrome (PHTS) due to elevated risk of thyroid carcinoma (TC). We aimed to evaluate the yield of TUS.

Materials and Methods: Adult PHTS patients who visited our expertise centre between 1997-2020 were included (N = 87). Data on TUS and clinical history were collected from medical records. Nodular progression was defined as an increase in the number and/or size of nodules or size of the thyroid gland.

Results: Within 75 patients, 310 yearly ultrasounds were performed with a total of 279 surveillance follow-up years. The median age at first TUS was 37 years (interquartile range (IQR): 24-43) with a median follow-up of 3 years (IQR: 1-5). At first TUS, 60/75 (80%) patients presented with multiple nodules. During follow-up, nodular progression was observed in 26/64 (41%) patients. Suspicious lymph nodes were found in 7/60 (12%) patients. Thyroiditis was diagnosed in 8/61 (13%) patients. Thyroidectomy was performed in 11/75 (15%) patients of which 7/11 occurred before initial TUS (all partial thyroidectomies). Reasons for surgery were suspicion of TC (N = 3) or multinodular goitre (N = 6). Four patients (median age of 24 years (IQR: 21-27)) presented with TC of which 3/87 had a medical history of TC and 1/75 developed TC during TUS. Histology showed 2 papillary and 2 follicular TCs.

Conclusions: Multinodular thyroid disease and thyroiditis are common findings in PHTS patients. The number of TCs is lower than what we had expected based on current TC risk estimates.

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P12.165.A A mosaic *PIK3CA* variant in a young adult with diffuse gastric cancer

Iris B. A. W. te Paske[1], *José Garcia-Pelaez*[2], *Anna K. Sommer*[3], *Leslie Matalonga*[4], *Teresa Starzynska*[5], *Anna Jakubowska*[6,7], *Solve-RD-GENTURIS group, Rachel S. van der Post*[8], *Jan Lubinski*[6], *Carla Oliveira*[2], *Nicoline Hoogerbrugge*[1], *Richarda M. de Voer*[1]

[1]*Department of Human Genetics, Radboud university medical center, Radboud Institute for Molecular Life Sciences, Nijmegen, Netherlands,* [2]*I3S, Instituto de Investigação e Inovação em Saúde, University of Porto, Porto, Portugal; Ipatimup, Institute of Molecular Pathology and Immunology of the University of Porto; Faculty of Medicine, University of Porto, Porto, Portugal,* [3]*Institute of Human Genetics, University of Bonn, Bonn, Germany,* [4]*CNAG-CRG, Centre for Genomic Regulation, Barcelona Institute of Science and Technology, Barcelona, Spain,* [5]*Department of Gastroenterology, Pomeranian Medical University, Szczecin, Poland,* [6]*Department of Genetics and Pathology, Pomeranian Medical University, Szczecin, Poland,* [7]*Independent Laboratory of Molecular Biology and Genetic Diagnostics, Pomeranian Medical University, Szczecin, Poland,* [8]*Department of Pathology, Radboud university medical center, Radboud Institute for Molecular Life Sciences, Nijmegen, Netherlands.*

Background: Hereditary diffuse gastric cancer (HDGC) is associated with germline pathogenic variants in *CDH1* and *CTNNA1*. However, a large proportion of clinically and pathologically HDGC-like families and individuals developing DGC at very young age remain genetically unresolved, raising the need for research on novel inherited predisposing factors. Under the collaborative environment of the SOLVE-RD consortium, we aimed to genetically diagnose these unresolved patients by re-analysis of their whole-exome sequencing data.

Methods: Whole-exome sequencing data from unresolved gastric cancer cases ($n = 83$) was processed by the RD-Connect Genome-Phenome Analysis Platform and annotated using the Ensembl Variant Effect Predictor algorithm. Variants present in genes associated with genetic tumor risk syndromes ($n = 229$) that were predicted by ClinVar as (likely) pathogenic were followed up for interpretation and validation.

Results: A mosaic pathogenic missense variant in *PIK3CA* was identified in a 25-year-old female with DGC without familial history for cancer. The variant, c.3140A>G (p.His1047Arg), was present at a low variant allele frequency (18%) in leukocyte-derived DNA. The variant was not found in our inhouse database nor in individuals from non-cancer SOLVE-RD cohorts. Somatic variants in *PIK3CA* are usually associated with overgrowth, a phenotype that was not observed in this patient.

Conclusions: This study highlights mosaicism as a potential -and understudied- mechanism in the etiology of DGC. Moreover, this case emphasizes the complexity of molecular diagnosis in genetic tumor risk syndrome suspected patients, as in many of these genetically unresolved patients an obvious genotype-phenotype correlation often cannot be found. *SOLVE-RD received funding from EU Horizon 2020 (No.779257)*

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P12.166.B Detection of PIK3CA mutations in breast cancer: comparison of next-generation sequencing and Sanger sequencing

Maria Michelli, *Eirini Roupou, Ilenia Chatziandreou, Alexandros Zougros, Angelica A. Saetta*

National and Kapodistrian University of Athens, Medical school, Athens, Greece.

Introduction: The PI3K pathway is well known for its role in cellular proliferation and survival. Mutations in PI3K p110α catalytic subunit (*PIK3CA* gene) are among the most common aberrations for breast cancer. *PIK3CA* mutations are currently a biomarker for personalized cancer therapy. Our aim was to evaluate the concordance between Next-generation sequencing (NGS) and Sanger Sequencing for mutation detection in exon 9 and 20 of *PIK3CA* gene.

Materials and Methods: We examined 115 breast carcinomas and 15 cfDNA samples. 85 of the samples were analyzed by both methods whereas 28 samples were analyzed only by Sanger due to inadequate DNA quality for NGS and 17 samples were analyzed only by NGS.

Results: *PIK3CA* mutations were found in 26% of the samples using NGS, and in 30% of the samples, using Sanger. The most frequent mutations were: p.H1047R in 42% of the cases and p. E545K in 22%. 21 samples gave non informative results (<100.00 reads) by NGS and in 6 of them mutations were detected using Sanger sequencing. In 3 mutant cases by NGS the mutations could not be detected by Sanger due to low variant frequencies. The concordance of the two methods was 95%.

Conclusions: A good concordance was found between the two methods; NGS shows a greater analytical sensitivity and Sanger sequencing is an alternative method for samples showing low DNA quality and quantity for NGS.

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P12.167.C Droplet digital PCR and RT-qPCR as tools for detections of *PIK3CA* mutations in head and neck squamous cell carcinoma

Edyta M. Borkowska[1], *Magda Baranska*[2], *Magdalena Kowalczyk*[2], *Wioletta Pietruszewska*[2]

[1]*Medical University of Lodz; Chair of Laboratory and Clinical Genetics, Department of Clinical Genetics, Lodz, Poland,* [2]*Medical University of Lodz; Department of Otolaryngology, Head and Neck Oncology, Lodz, Poland.*

Head and neck squamous cell carcinomas (HNSCC) are the 7th cause of human malignancy with low survival rate due to late diagnosis and treatment. Its etiology is diverse, however, genetic factors are significant. The most common mutations in HNSCC were found in genes: *PIK3CA* (10-12%), *BRCA1* (6%), and *BRCA2* (7-9%). In some cases, these biomarkers correlate with recurrences or survival showing a potential of prognostic and predictive value. 113 formalin-fixed paraffin embedded (FFPE) tumor samples were collected from patients with HNSCC (oropharynx: 65 (57.89%); larynx: 48 (42.11%)). We examined *PIK3CA* H1047R mutation by Real Time PCR (RT-qPCR) and droplet digital PCR (ddPCR). *BRCA1* and *BRCA2* mutations were analyzed by RT-qPCR whereas p16 protein expression was assessed by immunohistochemistry. Finally, we identified HPV infection by RT-qPCR. The relationships between genomic alterations and clinical parameters were assessed using the Yates' corrected Chi2 test or Fisher's exact test for nominal variables. Kaplan Meier plots were applied for survival analysis. Our results revealed 9 *PIK3CA* H1047R mutations detected by ddPCR: 8 of them were negative in RT-qPCR. No *BRCA1* or *BRCA2* mutations were detected in the examined group. Only clinical features (nodal involvement, tumor stage; $p < 0.001$), but not genetic markers were related to overall and recurrence-free survival. We confirmed usefulness of ddPCR in the PIK3CA mutation assessment in FFPE samples.

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P12.169.A Specifications of the ACMG/AMP variant interpretation guidelines for germline POLE and POLD1 variants

Pilar Mur[1,2], *Elsa Ezquerro*[1], *Sandra García-Mulero*[3,4], *Lorena Magraner-Pardo*[5], *Marta Pineda*[1,2], *Lidia Feliubadaló*[1,2], *Gabriel Capellá*[1,2], *Rebeca Sanz*[3,4], *Conxi Lázaro*[1], *Tirso Pons*[6], *Laura Valle*[1,2]

[1]*Hereditary Cancer Program, Catalan Institute of Oncology; Oncobell Program, IDIBELL, Hospitalet de Llobregat (Barcelona), Spain,* [2]*Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Madrid, Spain,* [3]*Unit of Biomarkers and Susceptibility, Cancer Prevention and Control Program, Catalan Institute of Oncology; Oncobell Program, IDIBELL, Hospitalet de Llobregat (Barcelona), Spain,* [4]*Centro de Investigación Biomédica en Red de Epidemiologia y Salud Pública (CIBERESP), Madrid, Spain,* [5]*Prostate Cancer Clinical Research Unit. Spanish National Cancer Research Center (CNIO), Madrid, Spain,* [6]*Department of Immunology and Oncology, National Center for Biotechnology (CNB-CSIC), Spanish National Research Council, Madrid, Spain.*

Intro: Germline pathogenic variants in the exonuclease domain of polymerases *POLE* and *POLD1* -affecting their proofreading activity-, predispose to adenomatous polyps, colorectal cancer (CRC), and endometrial and ovarian cancers, among other extracolonic malignancies. Challenges in their variant interpretation has been shown across clinical and research laboratories (>90% of exonuclease domain variants are of uncertain significance (VUS) or have conflicting results; ClinVar 2020). Here, we aim to establish gene-specific guidelines for the classification of germline *POLE* and *POLD1* variants, following the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) recommendations.

Material and methods: Specific pieces of evidence considering contact with DNA binding cleft, activity assays, in silico predictions, tumor mutational signatures, clinical phenotypes, frequency in controls, and cosegregation data, were evaluated for their inclusion in the ACMG/AMP guidelines. Critical review of the literature and analysis of 20 *POLE* and *POLD1* benign and pathogenic variants were performed, finally considering 30 variants with uncertain significance to assess the applicability of different rule codes.

Results: Specifications were developed for nineteen ACMG/AMP criteria while ten were not applicable. From a total of 50 variants considered, 10 were reclassified (2 (likely) pathogenic as VUS, 6 VUS as (likely) benign, and 2 VUS as (likely) pathogenic).

Conclusion: Use of *POLE* and *POLD1*-specific ACMG/AMP guidelines for germline variant classification led to a decrease in VUS and will improve the clinical and therapeutical management of variant carriers.

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P12.170.B Combination of an 18-SNP polygenic risk score and classical risk factors for the prediction of breast cancer risk in Cypriot women

Kristia Yiangou[1,2], *Maria A. Loizidou*[1,2], *Eleni Kakouri*[3], *Yiola Marcou*[3], *Kyriacos Kyriacou*[1,2], *Andreas Hadjisavvas*[1,2], *Kyriaki Michailidou*[2,4]

[1]*Department of Electron Microscopy/Molecular Pathology, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus,* [2]*The Cyprus School of Molecular Medicine, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus,* [3]*Bank of Cyprus Oncology Center, Nicosia, Cyprus,* [4]*Biostatistics Unit, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus.*

Introduction: A polygenic risk score (PRS) summarizes the combined effect of multiple common low-penetrant single nucleotide polymorphisms (SNPs), mainly identified through genome-wide association studies (GWAS), and has the potential to be used for the prediction of breast cancer risk. The combination of PRS with classical breast cancer risk factors can improve risk stratification and personalized preventative strategies.

Materials and Methods: We assessed the predictive ability of a multivariable model consisting of an 18-SNP PRS and classical breast cancer risk factors in Cypriot women using 1,109 cases and 1,177 controls from the MASTOS study. Logistic regression analysis was performed to evaluate the association between each risk factor and breast cancer risk and to assess for interactions. Hosmer-Lemeshow goodness-of-fit test and AUC were calculated to evaluate the accuracy and the predictive power of the models.

Results: The 18-SNP PRS was significantly associated with an increased breast cancer risk in Cypriot women. The multivariable model, which combines the effects of the classical breast cancer risk factors and the 18-SNP PRS achieved the highest risk discrimination score.

Conclusions: These results suggest that the multivariable model may be used in Cypriot women for breast cancer risk stratification and support its potential clinical utility for personalized breast cancer risk prediction.

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P12.171.C Differences between inherited and acquired polymerase proofreading deficiencies in cancer

Elsa Ezquerro[1], *Pilar Mur*[1,2], *Sandra García-Mulero*[3], *Lorena Magraner-Pardo*[4], *Rebeca Sanz-Pamplona*[3], *Tirso Pons*[5], *Gabriel Capellá*[1,2], ***Laura Valle***[1,2]

[1]*Hereditary Cancer Program, Catalan Institute of Oncology; Oncobell Program, IDIBELL, Barcelona, Spain,* [2]*Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Madrid, Spain,* [3]*Unit of Biomarkers and Susceptibility, Cancer Prevention and Control Program, Catalan Institute of Oncology; Oncobell Program, IDIBELL, Barcelona, Spain,* [4]*Prostate Cancer Clinical Research Unit, Spanish National Cancer Research Center (CNIO), Madrid, Spain,* [5]*Department of Immunology and Oncology, National Center for Biotechnology (CNB-CSIC), Spanish National Research Council, Madrid, Spain.*

Intro: Pathogenic variants in the exonuclease domain of *POLE* and *POLD1* affect their proofreading activity and promote tumorigenesis. Inherited polymerase proofreading deficiency cause an autosomal dominant cancer- and polyposis-predisposing syndrome. On the other hand, somatic POLE exonuclease domain mutations are identified in 7-15% of endometrial cancers, 0.5-8% of colorectal cancers, and more rarely in other tumors. We aimed to establish the differences between germline and somatic variants.

Material and methods: Analysis of *POLE/D1* exonuclease-domain pathogenic variants reported in the literature (15 germline variants in 59 independent families) and in publicly available databases (18 somatic variants in 77 proofreading-deficient tumors; source: TCGA), was performed. The variants were analyzed according to their nature (contact with DNA binding cleft, location within highly conserved motifs and pathogenicity predictions), tumor mutational characteristics and clinical phenotypes.

Results: *POLD1* somatic variants are extremely rare and, whenever present, appear in combination with mismatch repair deficiency. The spectrum of germline POLE proofreading mutations differs from the somatic mutation hotspots identified in tumors, suggesting a different behavior. Interestingly, when *POLE* p.V411L and p.A456P, two of the highly recurrent somatic variants, occur in the germline, the clinical phenotypes are extremely aggressive and precocious. Depending on the inherited or somatic nature of the variant, one residue may be mutated to generate one or other amino acid. When this occurs, the germline change shows lower pathogenicity prediction values that the somatic change.

Conclusion: Inherited pathogenic variants affecting the proofreading activity of polymerases are less aggressive that those identified in sporadic tumors.

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P12.172.D Searching for germinal mutations of TET2, KMT2D, KDM6B, IDH1 and SETD2 epigenetic genes in Polish prostate cancer patients - preliminary results

Marta Karolina Heise[1], *Piotr Jarzemski*[2], *Aneta Bąk*[1], *Anna Junkiert-Czarnecka*[1], *Maria Pilarska-Deltow*[1], *Maciej Borysiak*[2], *Beata Pilarska*[2], *Olga Haus*[1]

[1]*Department of Clinical Genetics, Faculty of Medicine, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University, Toruń, Poland,* [2]*Department of Urology, Jan Biziel University Hospital, Bydgoszcz, Poland.*

Introduction: The epigenetic changes are present in all human cancers and associated with genetic alterations to drive a cancer phenotype. Thus, we searched for germinal alterations in five epigenetic genes in Polish prostate cancer patients.

Material: The material of investigation was DNA from 27 men with prostate cancer (PC) from all over Poland. The median age of patients at PC diagnosis was 58 years (45-70).

Methods: NGS and Sanger sequencing.

Results: In 8/27 (29,6%) PC patients 9 variants of analyzed genes were detected. These were 8 missense mutations of *KMT2D*, *SETD2*, *KDM6B* and *IDH1* and one silent variant of *TET2*. Bioinformatic analysis of all changes was performed using VarSome database. The *KMT2D* c.13629C>A (p.Asp4543Gln), c.11375C>T (p.Pro3792Leu) and c.8788C>T (p.Pro2930Ser), the *IDH1* c.565A>G (p.Ile189Val) and the *KDM6B* c.2282C>G (p.Thr761Ser) missense variants were predicted as VUS (variants of uncertain significance). The *KMT2D* c.6264C>T (p.=) was predicted as benign and probably not involved in RNA splicing, *TET2* c.972A>G (p.=) as likely benign and probably involved in it. The *SETD2* c.3229A>G (p.Thr1077Ala) was predicted as benign and c.3383C>G (p.Thr1128Ser) as likely benign.

Conclusions: The results of the preliminary investigation point at the need to study germinal changes of epigenetic genes to help fully understand the pathogenesis of prostate cancer, identify men at PC high risk and predict the disease recurrence risk after radical prostatectomy. This study was supported by the fund of the Collegium Medicum Nicolaus Copernicus University, Bydgoszcz, Poland.

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P12.173.A "Next-Generation Sequencing to characterize tumor genomics in Prostate Cancer"

*Pamela Vázquez-Cárdenas[1,2,3], **Nuria Arroyo-Garrapucho**[1,2,3], Juan Luis García[2,3], Luis Angel Corchete[2,3], Pablo Berrocal-Navarro[1], Álvaro Jesus Virseda-Rodríguez[4], Sara Marcos-Asensio[4], Patricia Antunez[5], Ana Belen Herrero[1,2,3], Francisco Gómez-Veiga[4], Juan Jesus Cruz-Hernández[6,2], Rogelio González-Sarmiento[1,2,3]*

[1]Molecular Medicine Unit, Department of Medicine, University of Salamanca, Salamanca, Spain, [2]Biomedical Research Institute of Salamanca (IBSAL), University Hospital of Salamanca, Salamanca, Spain, [3]Institute of Molecular and Cellular Biology of Cancer (IBMCC), University of Salamanca-CSIC, Salamanca, Spain, [4]Translational Research Group of Urology GITUR-IBSAL, University Hospital of Salamanca, Salamanca, Spain, [5]Pathological Anatomy Service, University Hospital of Salamanca, Salamanca, Spain, [6]Oncology Service, University Hospital of Salamanca, Salamanca, Spain.

Introduction: Prostate adenocarcinoma (PCa) mainly affects older men and its incidence rapidly increases after the age of 50. However, the molecular and clinical behavior of PCa in younger adults is poorly defined. This study aims to identify variants associated with PCa and correlate them with clinical data in younger and older patients.**Patients and Methods**: A NGS target enrichment panel (CELEMICS) was used to analyze 4 genes (*SRD5A1*, *SRD5A2*, *NR3C1*, *AR*) associated with PCa. We included 8 paraffin-embedded samples derived from: 5 older patients diagnosed with castration resistant PCa and *de novo* metastatic PCa, and 3 younger patients with low-grade PCa. All samples were sequenced on the Illumina Miseq and the variants were analyzed using the VariantStudio Software.

Results: We have identified 5 missense, 1 nonsense, 1 frameshift mutation and 1 in-frame deletion. Two of these mutations were pathogenic and located in the *AR* gene in 2 patients: **c.2257C>T/p.(Arg753Ter)** in one *de novo* metastatic older patient and **c.2323C>T/p.(Arg775Cys)** in one younger patient. Two variants of uncertain significance were found in the *AR* gene.

Conclusion: Our results highlight that NGS panels allow for the identification of variants. This approach is an appropriate tool for understanding the difference between molecular profiles in younger and older patients with PCa.This study funded by Spanish Association Against Cancer (AECC).

		Sample ID									
Gene	**Variant**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Clasification**	**Clinical Significance**
SRD5A1	c.90C>G/p.(Ala39Gly)	+	+	+	+	+	-	+	+	*Missense*	Benign
SRD5A2	c.265C>G/ p. (Leu89Val)	-	-	-	-	-	+	-	-	*Missense*	Benign
SRD5A2	c.226T>A/ p. (Ser76Thr)	-	-	-	-	-	+	-	-	*Missense*	Not reported
SRD5A2	c.89dupC/ p. (Pro31Ser31fsTer225)	+	+	+	+	+	+	+	+	*Frameshift*	Benign
AR	c.2257C>T/p. (Arg753Ter)	-	-	+	-	-	-	-	-	*Nonsense*	**Pathogenic**
AR	c.2323C>T/p. (Arg775Cys)	-	-	-	-	-	+	-	-	*Missense*	**Pathogenic**
AR	c.404C>T/ p. (Pro135Leu)	-	-	-	-	-	+	-	-	*Missense*	Uncertain Significance
AR	c.170T>A/ p. (Leu57Gln)	-	-	-	-	+	-	-	-	*Missense*	Likely benign
AR	c.1368_1379del/p. (Gly470_Gly473del)	-	-	+	-	-	-	-	-	*In-frame Deletion*	Uncertain Significance

+: Presence, - : Absence

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P12.174.B Comparison of single nucleotide polymorphisms in early and advanced prostate cancer

***Justina Gaizevska**[1,2], Mantautas Simkus[2], Rasa Sabaliauskaite[1], Paulius Bosas[1], Arnas Bakavicius[1,3,4], Albertas Ulys[1], Feliksas Jankevicius[3,4], Sonata Jarmalaite[1,2]*

[1]National Cancer Institute, Vilnius, Lithuania, [2]Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania, [3]Urology Centre, Vilnius University Hospital Santaros klinikos, Vilnius, Lithuania, [4]Institute of Clinical Medicine, Faculty of Medicine, Vilnius University, Vilnius, Lithuania.

Introduction: Prostate cancer (PC) is one of the most common types of cancer. Despite the recent progress of diagnosis and research, it remains a significant medical problem. The attention is paid to the identification of genetic variation, which increases susceptibility because it could help to develop screening strategies and clinical management. Numerous single nucleotide polymorphisms (SNPs) that might play an aggregate role in PC susceptibility have recently been identified.

Materials and Methods: DNA was extracted from the leucocytes of patients whose cancer is in the early stages ($n = 128$) and castration-resistant prostate cancer (CRPC) ($n = 149$). qPCR with TaqMan assays were used to identify SNPs found in *HOXB13*, *KLK3*, *CDKN1B*, *RFX6* and *ANO7* genes. Associations between the form of cancer and clinical characteristics, genotypes and clinical characteristics were analysed.

Results: The genotypes of early-stage and CRPC patients in reference to the SNPs of five genes were identified. Significant associations between the form of cancer and patients' Gleason score, PSA level and the existence of metastasis were identified ($p < 0.001$). Association between the genotypes in terms of *KLK3* gene's SNPs and PSA level were statistically significant among early-stage cancer patients ($p = 0.036$, $p = 0.042$). A significant association between the genotypes regarding *ANO7* gene's SNP and metastasis to the axial skeleton was detected ($p = 0.024$).

Conclusions: Our study identified frequency of SNP in prostate cancer patients. We determined the associations between identified genotypes and analysed clinical characteristics.

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P12.175.C A case of PTEN hamartoma tumor syndrome; a family study

Hatice Ilgin-Ruhi[1], *Ezgi Gokpinar-Ili*[2], *Naz Guleray Lafci*[3], *Elifcan Tasdelen*[4], *Sule Altiner*[1]

[1]*Ankara University Medical Faculty, Ankara, Turkey,* [2]*Başakşehir Pine and Sakura City Hospital, Istanbul, Turkey,* [3]*Dr. Sami Ulus Maternity and Children's Health and Diseases Training and Research Hospital, Ankara, Turkey,* [4]*Sanliurfa Education and Research Hospital, Sanliurfa, Turkey.*

Introduction: Germline *PTEN* mutations are the cause of a group of cancer susceptibility syndromes such as Cowden and Bannayan-Riley-Ruvalcaba syndromes, that are within the PTEN hamartoma tumor syndrome (PHTS) spectrum. They have overlapping clinical findings and they are usually inherited autosomal dominantly. Here, we present a case with multiple abdominal subcutaneous nodular lesions and dysmorphic features.

Materials and Methods: A 15-year-old male patient was evaluated in terms of anamnesis, clinical history, family history and phenotypic findings. PTEN mutations were investigated in accord with PHTS pre-diagnosis.

Results: He was born to non-consanguineous parents. His prenatal and postnatal history were uneventful. Maternal grandmother died due to a brain tumor at the age of 47. He was operated for VSD at the age of two. Macrocephaly, thick and dry hair, low anterior hairline, upslanting palpebral fissures, low set ears, high arched palate, pes cavus, ulnar deviation of 1st toes were notable findings along with mild intellectual disability. There were extensive subcutaneous nodular lesions in the abdomen and legs. Germline *PTEN* mutations were investigated via Sanger sequencing and heterozygous *PTEN* c.-1196_-1185del12 promoter mutation was detected. Following genetic counseling, parental study was performed for segregation analysis and maternal inheritance was shown.

Conclusions: Although the detected mutation (rs587781340) is non-consensual in terms of clinical significance, promoter mutations are reported to cause PHTS. Hence, we planned oncological evaluation and follow-up of the proband and his mother. His brother and two maternal uncles were also invited for mutation analysis.

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P12.177.C *RAD51C* and *RAD51D* germline mutations are associated to susceptibility to multiple cancers

Jacopo Boni[1,2], *Aida Idani*[1,2], *Evan Weber*[3], *Eva Tomiak*[4], *William Foulkes*[5,6,7], *Zaki el Haffaf*[8], *Barbara Rivera*[1,2,7]

[1]*Program in Molecular Mechanisms and Experimental Therapy in Oncology (Oncobell), IDIBELL, Hospitalet de Llobregat (Barcelona), Spain,* [2]*Hereditary Cancer Programme, Catalan Institute of Oncology, IDIBELL, Hospitalet de Llobregat (Barcelona), Spain,* [3]*Division of Medical Genetics, Department of Specialized Medicine, McGill University Health Centre, Montreal, QC, Canada,* [4]*Department of Genetics, University of Ottawa, Children's Hospital of Eastern Ontario, Ottawa, ON, Canada,* [5]*Cancer Research Program, The Research Institute of the McGill University Health Centre, Montreal, QC, Canada,* [6]*Department of Human Genetics, McGill University, Montreal, QC, Canada,* [7]*Gerald Bronfman Department of Oncology, McGill University, Montreal, QC, Canada,* [8]*Division of Genetics, Department of Medicine, Centre hospitalier de l'Université de Montréal (CHUM), Montreal, QC, Canada.*

Introduction: Defects in DNA repair genes have been extensively associated to cancer susceptibility. In particular, pathogenic germline mutations in genes involved in homologous recombination repair pathway lead to a hereditary breast and ovarian cancer syndrome (HBOC). The RAD51 paralogs RAD51C and RAD51D were included in this group 10 years ago, when germline mutations in them were associated to non-BRCA1/2 familiar breast/ovarian cancer and ovarian cancer, respectively. However, whether pathogenic germline variants in these genes are associated to other cancers remains unknown.

Materials and Methods: We have systematically reviewed the landscape of *RAD51C* and *RAD51D* mutations in cancer by cataloguing reported variants in the literature during the last 10 years, and curating all mutations and phenotypes found in families with carriers of pathogenic *RAD51C/D* variants. Data were obtained from both published reports and in house samples from patients and families.

Results: A comprehensive catalogue of *RAD51C/D* variants has been generated. A total of 248 and 155 unique alterations in *RAD51C* and *RAD51D* have been described, respectively. Investigation of pedigrees found other cancers reported in families with *RAD51C/D* mutation carriers, with colorectal, lung, prostate, pancreatic cancer and leukemia being the most prevalent ones among first relatives.

Conclusions: Here we provide the first comprehensive catalogue of *RAD51C/D* pathogenic variants in cancer. Our works highlights how the two genes might confer susceptibility to a broader spectrum of cancer types than those characteristic of HBOC. Fundings. This research was partially funded by the Cancer Research Society grant OG-24377 to Barbara Rivera.

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P12.178.B Introduction of the renal cell carcinoma microarray service in the North of Scotland Genetics & Molecular Pathology Laboratory

Clare Wells, *Myra Loomer, Sinclair Dundas, David McClelland, Benedict Milner, Caroline Clark*

NHS, Aberdeen, United Kingdom.

Renal cell carcinomas (RCCs) are a highly heterogenous group of tumours derived from renal tubular epithelial cells, and together constitute the 7th most common cancer in the UK. Accurate classification of these tumours is essential to facilitate appropriate patient management. Diagnosis has historically relied upon immunohistochemistry and morphological assessment; however results from these techniques can often overlap between RCC subtypes, necessitating a more specific means of diagnosis.

Each of the three of the most common types of RCCs are associated with characteristic patterns of chromosomal aberrations: clear cell RCCs show loss of 3p, papillary cell RCCs exhibit gain of chromosomes 7, 8, 12, 16, 17 and 20 and loss of Y, and chromophobe RCCs are associated with loss of chromosomes, commonly 1, 2, 6, 10, 13, 17 and 21. Oncocytomas are benign renal lesions which can show morphological features similar to malignant RCCs, and are characterised by whole or partial loss of chromosome 1 and Y. Given the well-defined chromosomal aberrations recognised in these tumours, genetic analysis of RCCs can aid in their classification. The North of Scotland Genetics & Molecular Pathology Laboratory has offered fluorescent in situ hybridisation (FISH) testing on formalin fixed paraffin embedded (FFPE) RCC tumours for eight years. More recently, our laboratory have validated the ThermoFisher CytoScan 750k SNP microarray platform for genome wide analysis of FFPE renal tumours. This poster will demonstrate the utility of this powerful diagnostic tool as we review the initial phase of its introduction into service.

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P12.179.C Constitutional *POLE* variants known to be somatic driver mutations in cancer cause a phenotype reminiscent of Constitutional Mismatch Repair Deficiency

*Astrid Sehested[1], Julia Maede[2], David Scheie[3], Olga Østrup[4], Brigitte Bertelsen[4], Linea C. Melchior[3], Elena Kessler[2], Helga Fibiger Munch-Petersen[3], Tomasz Sarosiek[5], Reet Pai[6], Richard Gallon[7], Johannes Zschocke[8], **Katharina Wimmer**[8]*

[1]Dept of Pediatrics and Adolescent Medicine, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark, [2]Department of Pediatrics, Division of Pediatric Hematology/Oncology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA, [3]Department of Pathology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark, [4]Centre for Genomic Medicine, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark, [5]Luxmed Onkologia, Warsaw, Poland, [6]University of Pittsburgh Medical Center, Presbyterian Hospit, Pittsburgh, PA, USA, [7]Faculty of Medical Sciences, Newcastle University, Newcastle, United Kingdom, [8]Institute of Human Genetics, Medical University Innsbruck, Innsbruck, Austria.

Introduction: Deficiencies of polymerase proofreading (PP) or mismatch repair (MMR) are typically acquired somatically in neoplastic cells, but can also be constitutional conditions associated with rare cancer syndromes. Germline heterozygous *POLE* or *POLD1* pathogenic variants (PVs) cause PP associated polyposis (PPAP), presenting with colorectal adenomas and carcinomas in adulthood. Constitutional MMR deficiency (CMMRD), caused by germline bi-allelic PVs affecting one of four MMR genes, results in a high propensity for hematological, brain, intestinal tract, and other malignancies in childhood. Non-malignant clinical features, of which skin pigmentation alterations are the most prevalent, are found in nearly all CMMRD patients and are important diagnostic markers.

Results: In three cancer patients with a clinical presentation highly suggestive of CMMRD, we excluded CMMRD and identified a constitutional heterozygous *POLE* PV. These, and two additional *POLE* PVs identified in previously published CMMRD-like patients, have not previously been reported as germline PVs causing PPAP but are all well-known somatic driver-mutations in hyper-mutated tumors.

Conclusions: Together these five cases show that constitutional heterozygous *POLE* driver-mutations cause a phenotype distinct from PPAP but resembling CMMRD due to the associated childhood/adolescent malignancies and non-malignant features. Therefore, a severe constitutional PP deficiency caused by de novo *POLE* variants, which may have a stronger "mutator" effect than *POLE/D1* PV causing PPAP, should be considered as a differential diagnosis to CMMRD. The common underlying mechanism, i.e. a replication error repair defect, and a similar tumor spectrum provides a good rationale for monitoring of these patients according to protocols proposed for CMMRD.

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P12.181.A cf-DNA and EVs as sources for biomarkers for early detection of second primary malignancies in patients with heritable retinoblastoma

***Nicole Barwinski**[1,2], Michael Zeschnigk[1], Basant Kumar Thakur[2], Petra Ketteler[1,2], Dietmar Lohmann[1]*

[1]Institute of Human Genetics, University Duisburg-Essen, Essen, Germany, [2]Department of Pediatric Hematology and Oncology, University Hospital Essen, University Duisburg-Essen, Essen, Germany.

Heritable retinoblastoma (Rb) is an autosomal-dominant tumour predisposition syndrome caused by pathogenic variants of the *RB1* gene. Rb-survivors have a high risk to develop a second primary malignancy (SPM). Development of retinoblastoma is often initiated by genetic mechanisms that result in loss of heterozygosity (LOH). It is to be expected that LOH is frequent in SPMs as well.

The aim of this study is to develop a non-invasive blood test for early detection of SPMs in Rb-survivors. Blood plasma-derived EVs, EV-DNA and cfDNA are analysed in blood samples from children with Rb and Rb-survivors with and without SPM. DNA released by tumour cells with LOH at the RB1 locus is expected to skew the ratio of *RB1* alleles in EV-DNA and cfDNA.

Using Droplet Digital PCR, the number of genome equivalents (GEs) was determined in EV-DNA (10 ± 9) and cfDNA (1584 ± 946). With numbers of GEs in this range, multiple informative SNPs need to be analysed to increase the power of detecting significant skewing of allele ratios in individuals with SPM compared to balanced ratios expected in healthy individuals.

The allelic ratio of multiple SNPs is determined by parallel analysis with Deep Amplicon Next-Generation Sequencing of cfDNA. To improve analytical accuracy The SiMSen-seq technology was chosen (Ståhlberg et al. 2017). By incorporating unique molecular identifiers, this technology reduces overestimation of sample size because of PCR inflation and, consequently under-estimation of variance.

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P12.182.B Phenotypic analysis of 106 serrated polyposis patients

***Verena Steinke-Lange**[1,2], Anna Lausch[2], Monika Morak[1,2], Elke Holinski-Feder[1,2]*

[1]MGZ - Medical Genetics Center, München, Germany, [2]Arbeitsgruppe erbliche gastrointestinale Tumore, Medizinische Klinik und Poliklinik IV – Campus Innenstadt, Klinikum der Universität München, Munich, Germany.

Background: Genetic and/or non-genetic causes of serrated polyposis syndrome (SPS) are widely unknown. SPS patients show a broad phenotypic spectrum regarding polyp burden and age of onset and might therefore belong to different entities. We therefore collected phenotypic data of 106 SPS patients to identify phenotypic subgroups.

Methods: 70 female (66 %) and 36 male patients were enrolled, 53 patients (50 %) fulfilled the WHO criteria for SPS. We calculated the annual gain of serrated polyps and compared the results depending on different parameters using Mann-Whitney-U tests.

Results: Female patients developed significantly more sessile-serrated lesions per year than male patients ($U = 885.500$; $p = 0.012$). This also applied for sessile-serrated lesions of the proximal colon ($U = 733.500$; $p = 0.0004$), but not for the distal colon ($U = 1158.00$; $p = 0.474$). Regarding hyperplastic polyps, there was no significant difference. Smoking and BMI also had no significant influence on serrated polyp burden in our cohort. Control colonoscopy 1-2 years after initial diagnosis revealed polyps in

70%. We were able to figure out two clinical subgroups: while one part of the patients was continuously prone to serrated polyps, the other patients only showed a temporary polyposis with several inconspicuous colonoscopies afterwards. Hypothetically, the first could be due to a genetic predisposition, while the other might mainly be exogenic. Only two patients developed colorectal cancer during surveillance, each after 4-5 years without colonoscopy.

Conclusions: SPS patients may either have a temporary or permanent risk for serrated polyps and colorectal cancer. Therefore, close meshed surveillance by colonoscopy especially following initial diagnosis is necessary.

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P12.183.C Exome sequencing identified potential causative candidate genes for serrated polyposis syndrome

Sophia Peters[1], *Anna K. Sommer*[1], *Christina Trueck*[1], *Verena Steinke-Lange*[2,3], *Claudia Perne*[1,4], *Janine Altmueller*[5], *Holger Thiele*[5], *Elke Holinski-Feder*[2,3], *Isabel Spier*[1,4], *Stefan Aretz*[1,4]

[1]*Institute of Human Genetics, Medical Faculty, University of Bonn, Bonn, Germany,* [2]*MGZ-Medical Genetics Center Munich, Munich, Germany,* [3]*Arbeitsgruppe erbliche gastrointestinale Tumore, Medizinische Klinik und Poliklinik IV – Campus Innenstadt, Klinikum der Universität München, Munich, Germany,* [4]*National Center for Hereditary Tumor Syndromes, University Hospital Bonn, Bonn, Germany,* [5]*Cologne Center for Genomics, University of Cologne, Cologne, Germany.*

Aim: Serrated polyposis syndrome (SPS) is a poorly defined colorectal cancer predisposition syndrome characterized by multiple and/or large serrated lesions throughout the colon. To date, only few molecular signatures have been described and the etiology of the syndrome has not been identified in the vast majority of patients. The aim of this study is to identify causal germline variants for SPS.

Methods: To uncover predisposing causative variants, the exomes of 102 unexplained SPS patients have been sequenced using leukocyte DNA. For data analysis and filtering, the GATK software and the Varbank2 software were applied. The germline variants were filtered for rare (allele frequency for biallelic ≤ 1%, for monoallelic ≤ 0.1% according to gnomAD and an in-house database) loss-of-function and missense variants with a CADD-score ≥ 20.

Results: After filtering and manual inspection, 495 genes harboring rare heterozygous variants in at least two patients remained; potentially biallelic variants were found in 189 genes. After prioritizing the genes, promising candidate genes for SPS that harbor potential biallelic variants include among others *ANKRD17, LAMA5* as well as *MCM3* while genes that harbor heterozygous variants include *CDKN1A, CEACAM1*, and *PTPRT*.

Conclusions: Exome sequencing identifies potentially causative germline variants underlying the susceptibility to SPS; however, the preliminary data indicate considerable genetic heterogeneity. The current work-up includes testing of relatives to determine the zygosity of assumed biallelic variants, analyzing the segregation with the phenotype, and functional analyses of the most interesting variants. Additionally, a burden test will be conducted.

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P12.185.A Cancer spectrum and penetrance in a national cohort of patients with a loss-of-function germline *SMARCA4* alteration

Nienke van Engelen[1], *Jozef Zsiros*[1], *Saskia M. J. Hopman*[2], *M C. A. Cornips*[2], *Nienke E. Verbeek*[2], *Carli M. J. Tops*[3], *Maartje Nielsen*[3], *Dennis K. Bos*[4], *Encarna B. Goméz-García*[5], *Arjen R. Mensenkamp*[6], *Charlotte W. Ockeloen*[6], *Rolph Pfundt*[6], *Lynne Rumping*[7], *Anne Goverde*[8]

[1]*Princess Maxima Center for Pediatric Oncology, Utrecht, Netherlands,* [2]*University Medical Center Utrecht, Utrecht, Netherlands,* [3]*Leiden University Medical Center, Leiden, Netherlands,* [4]*University Medical Center Groningen, Groningen, Netherlands,* [5]*Maastricht University Medical Center+, Maastricht, Netherlands,* [6]*Radboud University Medical Center, Nijmegen, Netherlands,* [7]*Amsterdam Medical Center, Amsterdam, Netherlands,* [8]*Erasmus Medical Center, Rotterdam, Netherlands.*

Introduction: Loss-of-function germline *SMARCA4* variants predispose to rhabdoid tumors in children and small cell carcinoma of the ovary hypercalcemic type (SCCOHT) in girls and women. Cancer penetrance is unknown, which complicates the development of surveillance and prevention guidelines. We therefore describe a national cohort of individuals carrying germline *SMARCA4* alterations and their cancer phenotype.

Methods: We have collected clinical and genetic data from individuals with a germline loss-of-function *SMARCA4* alteration through all Dutch DNA diagnostic laboratories.

Results: We have identified 16 individuals from 12 families. In 5 probands the *SMARCA4* variant was detected after cancer diagnosis (4 SCCOHTs, 1 rhabdoid tumor). One patient has inherited the variant from her mother and grandmother, neither of whom developed *SMARCA4*-related cancers. Five probands (mean age 28 years [range 6-51 years] 4 females), all without cancer, carry a *de novo* 19p13.2 deletion including *SMARCA4* and are affected by developmental delay. Finally, in two probands and two of their relatives (mean age 33 years [range 15-54 years] 3 females), also all without cancer, the *SMARCA4* variant was an incidental finding.

Conclusion: Our data reveal an incomplete penetrance for cancer in individuals with germline loss-of-function *SMARCA4* alterations and suggest a possibly lower penetrance in patients with multigene deletions spanning *SMARCA4*. We hypothesize that these larger deletions include additional genes essential for cell survival. Affected cells might not survive in a homozygous deleted state, therefore prohibiting tumor development due to a large deletion affecting the wild type allele, a common somatic alteration in *SMARCA4*-related tumors.

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P12.186.B SOX3 function in glioblastoma cells

Jelena Marjanovic[1], ***Danijela Drakulic***[1], *Idoia Garcia*[2,3,4], *Vladanka Vukovic*[1], *Paula Aldaz*[2,4], *Laura Garros-Regulez*[2], *Nicolas Sampron*[2,4,5], *Ander Matheu*[2,3,4], *Milena Stevanovic*[1,6,7]

[1]*Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia,* [2]*Cellular Oncology group, Biodonostia Health Research Institute, San Sebastian, Spain,* [3]*IKERBASQUE,*

Basque Foundation for Science, Bilbao, Spain, [4]CIBER de Fragilidad y Envejecimiento Saludable (CIBERfes), Madrid, Spain, [5]Neuro-oncology Tumor Board, Donostia Hospital, San Sebastian, Spain, [6]University of Belgrade, Faculty of Biology, Belgrade, Serbia, [7]Serbian Academy of Sciences and Arts, Belgrade, Serbia.

Introduction: Glioblastoma (GBM) represents significant public health problem. Median survival time of patients with this type of brain tumor is about 15 months despite current therapeutic strategies including surgery, radiotherapy and chemotherapy. Thus, searching for new therapeutic treatments for GBM is warranted. Identification and characterization of molecular markers might lead to identification of specific targets for treatment of patients with GBM. SOX3 transcription factor is key regulator of cell fate decisions in numerous developmental processes. Oncogenic activity of SOX3 has been demonstrated in different cancer types. However, little is known about its function in GBM.

Materials and Methods: SOX3 expression in human GBM samples, non-tumoral brain tissues and GBM cells was assessed using RT-qPCR. To analyze the effects of SOX3 overexpression on the proliferation, viability, migration and invasion of GBM cells, immunocytochemistry, MTT and Transwell assays were employed, respectively. RT-qPCR and Western blot analyses were used to analyze expression of components of Hedgehog signaling pathway and autophagy markers, respectively.

Results: SOX3 expression was elevated in GBM samples, patient-derived glioblastoma stem cells and oncospheres derived from glioblastoma cell lines. SOX3 overexpression led to increase in the proliferation, viability, migration and invasion of GBM cells, enhanced activity of the Hedgehog signaling pathway and suppressed autophagy.

Conclusions: SOX3 could be considered as potential molecular target in GBM.This work was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia (Grant No: 173051 and Grant No: 451-03-68/2020-14/200042) and by the Serbian Academy of Sciences and Arts (Grant No: F 24).

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P12.187.C The molecular consequences of the novel speckle-type POZ protein (*SPOP*) gene mutations at the protein level: A prostate cancer perspective

***Isil Ezgi Eryilmaz**, Berna Aytac Vuruskan, Onur Kaygısız, Gulsah Cecener, Unal Egeli, Hakan Vuruskan*

Bursa Uludag University, Bursa, Turkey.

Introduction: *SPOP* encodes a substrate-binding subunit of a Cullin-based E3 ubiquitin ligase, which acts as a tumor suppressor by proteasomal degradation of oncogenic targets. The subunit is composed of the two main domains, MATH, and BTB/POZ. The overall frequency of *SPOP* variations ranged from 1.85% to 28.6% in multiethnic prostate cancer (PCa) cohorts. All of the variations have been reported in the MATH domain, suggesting that the variations disrupt the substrate interaction. However, we identified two novel *SPOP* variations localized in the BTB/POZ domain in three PCa patients (3.4%). The consequences of the variations were *in silico* analyzed, and the possible effects were discussed at the protein level.

Materials and Methods: Their effects on protein structure, stability, and function were tested using I-Mutant 2.0 and Mutation Taster tools. The 3D structures of the variant proteins were constructed by the Swiss-Model.

Results: The *SPOP* C203Y and S236R pathogenicities were 93% and 90%, respectively. Both variants were detected to be disease-causing and to cause a large decrease in protein stability with DDG values of -0.08 and -0.00 Kcal/mol. Moreover, the protein feature and function might be affected via loss of the BTB domain from the start point-173 to end point-297, visualized by 3D constructions of the variants.

Conclusions: This is the first report to identify the novel and disease-causing SPOP variations in a Turkish PCa cohort. Further studies should focus on functional analysis of these variants that might disrupt the ligase activity due to the loss of the BTB/POZ domain of SPOP.

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P12.188.D Efficient workflow for detection of clinically relevant abnormalities in leukemias according to NCCN guidelines

***Alex Hastie**, Andy W. C. Pang, Joyce Lee, Ernest Lam, Mark Oldakowski, Alka Chaubey*

Bionano Genomics, San Diego, CA, USA.

Current diagnostic and prognostic genetic testing for leukemias relies largely on cytogenetic methods. Cytogenetic testing involves 2-3 methods including karyotyping, a panel of fluorescent in situ hybridization (FISH) probes, and chromosomal microarray. Together these assays are cumbersome, low resolution, expensive, and require specialized, highly trained operators. Optical genome mapping (OGM) provides a modern solution to detect all clinically significant abnormalities using a single standardized workflow. We demonstrate a workflow for DNA isolation from bone marrow aspirates or peripheral blood, data collection, variation/abnormality calling, and annotation. DNA isolation via the Bionano Prep SP involves measuring 1.5 M cells from bone marrow or peripheral blood, lysing the cells, binding DNA to a paramagnetic disk, washing and eluting the DNA, this process takes 3-4 hours. The full process of DNA isolation, labeling, data collection, and SV calling takes four days total turn-around-time. We have evaluated system performance for detection of abnormalities according to the National Comprehensive Cancer Network (NCCN) guidelines for AML, CML, ALL, CLL, and MM patient samples. OGM is able to detect all cytogenetic level copy number variants and structural variants that are recommended by NCCN at variant allele fractions in the 5-10% range of somatic mosaicism, for example, BCR/ABL1, IGH/CCND1, RUNX1/RUNX1T1, IGH/IL3, Del6q, trisomy 12, 2p+, PML/RARA, MLL (KMT2A), ATM/TP53, monosomy 7. Taken together, optical genome mapping is able to provide results concordant with the combination of karyotyping and FISH, while providing the results in a single assay and with higher sensitivity.

A. Hastie: A. Employment (full or part-time); Significant; Bionano Genomics. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Bionano Genomics. **A.W.C. Pang:** A. Employment (full or part-time); Significant; Bionano Genomics. **J. Lee:** A. Employment (full or part-time); Significant; Bionano Genomics. **E. Lam:** A. Employment (full or part-time); Significant; Bionano Genomics. **M. Oldakowski:** A. Employment (full or part-time); Significant; Bionano Genomics. **A. Chaubey:** A. Employment (full or part-time); Significant; Bionano Genomics.

P12.190.B Tissue-specific patterns of mutational and transcriptional alterations in tumor suppressors and oncogenes: an integrative pan-cancer analysis

Andrea Gelemanovic, *Tinka Vidovic, Branka Bernard, Miroslav Radman, Katarina Trajkovic*

Mediterranean Institute for Life Sciences (MedILS), Split, Croatia.

Introduction: Although evident that some tissues are more susceptible to develop cancer than others, the biological basis of this tissue variability remains a challenge in cancer research. Here, we aim to characterize, at the level of mutations and transcriptomics, the tissue-specific patterns of two key players in the cancer initiation – activation of oncogenes and inactivation of tumor suppressors (TS).

Methods: We performed a pan-cancer differential gene expression analysis for nine cancer types (bladder, breast, colon, esophagus, kidney, liver, lung, stomach, thyroid gland) using the Cancer Genome Atlas (TCGA) and Genotype Tissue Expression (GTEx) adjusting for the effects of age and gender. Transcriptomic signatures of TSs and oncogenes were compared between the tissues and correlated with their respective cancer mutational burden.

Results: Similar trend was observed among tissues with larger numbers of overexpressed oncogenes and of underexpressed TSs. Exception were esophagus, lung and liver which displayed both more overexpressed TSs and oncogenes. Majority were tissue-specific, and only 8 TSs were underexpressed and 13 oncogenes were overexpressed across all tissues. Kidney exhibited a distinct transcriptomic signature. All tissues had more mutations in TSs than in oncogenes, and overall a low mutational burden, although in a highly tissue-specific pattern. Lung had the highest number of mutations with PCDH9 most frequently mutated (~6% of cancer samples).

Conclusions: This comprehensive analysis of mutational and transcriptional alterations in oncogenes and TSs in carcinogenesis revealed high tissue specificity and a low mutational burden, implying that epigenetic changes and/or strong selection might participate in tissue susceptibility to cancer.

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P12.191.C *TP53* gene alone and combination with others oncogenes hotspot mutations in NSCLC tumor and plasma samples of female

Ieva Drejeriene[1,2], *Arnoldas Krasauskas*[1,3], *Saulius Cicenas*[1,3], *Jurate Gruode*[2]

[1]*Vilnius University, Vilnius, Lithuania,* [2]*Klaipeda University Hospital, Klaipėda, Lithuania,* [3]*National Cancer Institute, Vilnius, Lithuania.*

Objectives: The aim of this study was to assess non-small cell lung cancer (NSCLC) female patients' hotspot mutations in oncogenes in formalin-fixed, paraffin-embedded (FFPE) tumor DNA and plasma cfDNA samples.

Materials and Methods: 49 female patients with NSCLC were included in study. The main morphology was adenocarcinoma - 36 (74%). Hotspot mutations in 22 oncogenes *(KRAS, EGFR, BRAF, PIK3CA, AKT1, ERBB2, PTEN, NRAS, STK11, MAP2K1, ALK, DDR2, CTNNB1, MET,* ***TP53****, SMAD4, FBX7, FGFR3, NOTCH1, ERBB4, EGFR1, FGFR2)* were detected using NGS (Ion Torrent™ PGM) Ion AmpliSeq colon and lung cancer research panel (ThermoFisher). NGS was performed from FFPE DNA and plasma cfDNA for every patient. Samples were taken before treatment.

Results: Mutations in *TP53* gene was the most prevalent in female tumor and plasma samples. In 49 FFPE and 49 plasma females' patients' samples was tested and results show 38 (78%) tumors and 10 (20%) plasma samples had detections one or more mutations in oncogenes. *TP53* gene mutations in tumor samples were detect 25 (43%) cases: 14 instances with other oncogenes mutations and 9 instances mutation alone in *TP53* gene; whereas, in plasma samples were detect 5 (38%) times: 3 instances with other oncogene mutations and 2 instances only mutation in *TP53* gene

Conclusion: Mutations in *TP53* gene are most prevalent in NSCLC tested group of samples. Although *TP53* gene is not related with lung cancer target therapy yet, identification presents and quantity of these mutation in plasma NSCLC patients can be used as a tool for follow up patients.

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P12.192.D Screening for genetic modifying factors in Li-Fraumeni and heritable *TP53*-related cancer syndromes

Gaelle Bougeard[1], *Sophie Coutant*[1], *Camille Charbonnier*[1], *Jacqueline Bou*[1], *Françoise Charbonnier*[1], *Emilie Bouvignies*[1], *Edwige Kasper*[1], *Stéphanie Baert-Desurmont*[1], *Claude Houdayer*[1], *Bertrand Fin*[2], *Anne Boland*[2], *Jean-François Deleuze*[2], *Laurence Brugières*[3], *Isabelle Tournier*[1], *Thierry Frebourg*[4]

[1]*Department of Genetics, Rouen University Hospital and Inserm U1245, Normandie University, Rouen, France,* [2]*Centre National de Recherche en Génomique Humaine, CEA, Université Paris-Saclay, Evry, France,* [3]*Department of Pediatric Oncology, Gustave Roussy, Villejuif, France,* [4]*Department of Genetics, Rouen University Hospital and Inserm U1245, Normandie University, ERN GENTURIS, Rouen, France.*

Penetrance of germline *TP53* variants is quite variable even within families. We performed an extensive comparison of the exome in 253 *TP53* variant carriers unaffected in childhood (uac) and in 174 *TP53* variant carriers affected in childhood (ac), including 62 with soft-tissue sarcoma or osteosarcoma (STS-OS), 50 with adrenocortical carcinoma, 20 with choroid plexus carcinoma and 42 with other malignancies. Analysis was performed at the exome level and on a panel of 688 genes including 296 DNA repair genes, 131 cancer predisposition genes (CPG), and genes involved in p53 pathway or drug metabolism. Screening for recurrent additional rare loss of function, strictly or moderately damaging missense variants did not reveal a significant enrichment of variants hitting a specific gene in the 174 ac, as compared to the 253 uac. We observed between both groups a similar number of variants *per* patient affecting the 688 genes (6 vs 6.25), 296 DNA repair genes (2.8 vs 2.9) and 131 CPG (1.57 vs 1.58) and did not observe in ac an enrichment of variants hitting these genes, as compared to the rest of the exome. Analysis of the ac series by tumour type did not show different numbers of variants. However, the pattern in ac with STS-OS was different from the others, with a predominance of variants affecting a gene controlling the mesenchymal state. This study shows that the mechanisms underlying the penetrance of germline *TP53* variants are complex and suggests that the tumour type depends on the genetic landscape.

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P12.193.A Inhibitors of TRAIL-induced apoptotic pathway: a study of relative mRNA expression patterns in breast tumors

Eirini Roupou[1], *Maria Michelli*[1], *Ilenia Chatziandreou*[1], *N.V. Michalopoulos*[2], *Panayiotis Karathanasis*[3], *Andreas C. Lazaris*[1], *Angelica A. Saetta*[1]

[1]*1st Department of Pathology, School of Medicine, National and Kapodistrian University of Athens, Ath, Athens, Greece,* [2]*Department of Surgery Attikon Hospital, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece,* [3]*1st Department of Propaedeutic Surgery Hippokrateion Hospital, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece.*

Introduction: TRAIL-induced apoptotic pathway is a promising therapeutic option as it targets cancer cells with high selectivity in vivo but the efficacy of TRAIL targeted monotherapies/combination therapies in clinical trials did not meet the expectations. Elucidating the expression patterns of pathway's inhibitors may aid in the selection of suitable cancer patients who would benefit most from the above mentioned targeted therapies.

Materials and Methods: Relative mRNA expression of TRAIL pathway inhibitors (*DcR1, DcR2, cFLIP, XIAP, BCL2, BCL-XL, MCL1)* was evaluated in 90 breast cancer tissues, using the RT-PCR/ΔΔCt method. The SPSSv22 package was used for statistical analysis.

Results: The pathway inhibitors presented elevated mRNA levels in 7%-18% of the cases and reduced mRNA levels in 27%-59% of the cases. Analysis of the simultaneous gene expression revealed linear correlations among different inhibitor pairs, with the strongest ones between MCL1/cFLIP ($R = 0{,}741$, $p < 0{,}001$) and MCL1/BCL-XL ($R = 0{,}714$, $p < 0{,}001$). The presence of lymph node metastasis correlated with *cFLIP* and *BCL-XL* expression ($p = 0{,}024$ and $p = 0{,}042$, respectively), pStage correlated with *cFLIP* and *MCL1* expression ($p = 0{,}046$ and $p = 0{,}041$, respectively) and the presence of a PIK3CA mutation correlated with *cFLIP* and *XIAP* expression ($p = 0{,}048$ and $p = 0{,}018$, respectively).

Conclusions: In our study, the expression of inhibitors of TRAIL apoptotic pathway depicted a complex regulation mechanism. Our analysis revealed correlations among inhibitors' relative mRNA levels with clinicopathological characteristics and multiple patterns of simultaneous expression. Considering the above, it is of significant importance to stratify breast cancer patients using predictive biomarkers in order to maximize the efficacy of TRAIL targeting therapies.

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P12.194.B Exploring Transposon Activity in Hematological Malignances

Anastasiya Volakhava*, Sarka Pavlova, Marcela Krzyzankova, Karol Pal, Hana Synackova, Alexander Komkov, Sarka Pospisilova, Ilgar Mamedov, Karla Plevova*

CEITEC, Brno, Czech Republic.

Transposable elements are repetitive mobile DNA sequences with the ability to invade and move within genomes. In the human genome, the vast majority of transposons is represented by retroelements (REs) that are categorized into several families. The long interspersed nucleic elements (L1) utilize a "copy-and-paste" mechanism to retrotranspose their copies into new genomic loci through RNA-mediated mechanisms. A subgroup of the short interspersed elements called Alu is nonautonomous and relies upon L1-encoded proteins (ORF1 and ORF2) for their mobilization. RE activity is one of the most important causes of genome instability. Somatic insertions were detected in cancer types, such as colorectal, lung, or breast carcinomas. To our best knowledge, no systematic analysis has been performed to date to study RE activity in hematological malignancies. We aimed to explore RE activity in chronic lymphocytic and acute lymphoblastic leukemias, and myelodysplastic syndrome. To identify tumor-specific RE insertions, we adopted an NGS protocol of amplicons containing a part of RE from Alu-Ya5, Alu-Yb8, or L1-HS families (the most active in humans), and its adjacent genomic region. In total, 118 samples (73 tumor and 45 normal DNA) from 49 patients were analyzed. We found 26 candidate insertions in 13 tumor samples that will be validated using PCR and Sanger sequencing. Additionally, to study RE activity on the protein level, we implemented Western blot analysis of ORF1 protein expression. In several cell lines, we observed ORF1p expression induction after azacytidine treatment causing genome demethylation. Supported by GACR 19-11299S, AZV NU21-08-00237, and MH-CZ RVO 65269705.

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P12.195.C Triple Negative Breast Cancer Risk Identified by Ondemand Gen Panel Testing

Mercedes Durán[1], *Mónica Arranz*[1], *Enrique Lastra*[2], *Luis Abella*[3], *Noemí Martínez*[1], *Lara Hernández*[1], *Mar Infante*[1]

[1]*IBGM, Valladolid, Spain,* [2]*Hospital Universitario de Burgos, Burgos, Spain,* [3]*Hospital Universitario Río Hortega, Valladolid, Spain.*

Introduction: Germline genetic testing with gene panels can identify women at increased risk of breast cancer. However, those at increased risk of triple-negative (estrogen receptor-negative, progesterone receptor-negative, human epidermal growth factor receptor-negative) breast cancer (TNBC) cannot be identified because predisposition genes for TNBC, other than BRCA1, have not been established. The aim of this study was to define new genes associated with increased risk of TNBC.

Materials and Methods: We designed an On-Demand panel for the analysis of 35-genes associated with inherited cancer susceptibility. 48 TNBC (BRCA1 negative) patients was performed. Each DNA sample was checked for concentration using a Qubit 3.0 Fluorometer. The library and template preparations were performed using the automated Ion Chef System, then sequenced in Ion S5 with Ion 520 Chip (all Thermo Fisher Scientific) according to the manufacturer's instructions. Sequencing results were analyzed using the Ion Reporter Software.

Results: A total of 3 Pathogenic or Likely Pathogenic variants (PV/LPV) were identified in 48 TNBC cases (6,3%), affecting 3 different genes with a current clinical utility for each tumor (Table).

Gene	cDNA	Protein	Consequence
ATM	c.5979_5983delTAAAG	p.Ser1993Argfs	Frameshift
BLM	c.1642C > T	p.Gln548Ter	Nonsense
BRIP1	c.206-2A > G	–	Splicng

Conclusions: This study identifies several genes that predispose to TNBC and are associated with high lifetime risks of TNBC and overall breast cancer. The implementation of gene-panels can improve the clinical management of TNBC patients in a quick and cost-effective method and the development of targeted therapeutic approaches for TNBC patients.

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P12.196.D mesenchymal stem cell-derived exosomes promote epithelial-to-mesenchymal transition in triple negative breast cancer cells

Ancuta Jurj[1], *Olga Soritau*[2], *Lajos Raduly*[1], *Cristian Moldovan*[1], *Cornelia Braicu*[1], *Ioana Berindan-Neagoe*[1]

[1]*Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania,* [2]*Laboratory of Radiotherapy, Tumor and Radiobiology, Prof. Dr. Ion Chiricuță Oncology Institute, Cluj-Napoca, Romania.*

Objective: Exosomes derived from mesenchymal stem cells (MSC) are critical players in the tumor niche being implicated in cell-to-cell communication affecting several hallmarks of cancer. The aim of this study was to investigate the influence of MSCs on triple negative breast cancer (TNBC) cell lines.

Methods: The TNBC cell lines were represented by MDA-MB-231 and Hs578T, while MSCs were primary cell cultures. Exosomes were isolated using ultracentrifugation and were characterized using the Nano Sight system. Cell viability was detected using the MTT assay while migration was analyzed through wound healing assay. Moreover, we also used 3D culture to assess the exosomes uptake and to observe their capability of internalization into a 3D structure. The alterations in expression level of some transcripts (mRNAs and miRNAs) were investigated by qRT-PCR and immunofluorescence.

Results: We observed that MSCs-derived exosomes were incorporated in the TNBC cell lines. Considering coculture conditions, in TNBC cells the expression level of mesenchymal markers and epithelial-to-mesenchymal transition (EMT) markers at mRNA and miRNA levels were significantly affected. Using bioinformatics tools, we highlighted the important altered pathways involved in EMT. In addition, using 3D culture we provided a comprehensive understanding regarding exosome internalization in 3D structures.

Conclusion: In the current study we have shown that MSC-derived exosomes alter the EMT in TNBC cell lines and that these alterations take place in a spatial-directed manner. Acknowledgement: PN-III-P1-1.2-PCCDI-2017-0782, entitled "Advanced innovative approaches for predictive regenerative medicine"—REGMED

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P12.197.A Analysis of urothelial carcinomas by targeted RNA-Seq

Jan Rehker, *Marie-Lisa Eich, Sabine Merkelbach-Bruse, Reinhard Büttner*

University Hospital Cologne, Cologne, Germany.

Urothelial carcinomas are the 9th most common type of cancer worldwide. Prognosis for outcome of the disease and treatment are associated with histopathology of the tumor. We analyzed a total of 36 samples by targeted RNA sequencing. 9 of the tumors were low grade (LG), 10 were high grade (HG) and 17 were high grade tumors that had invaded muscular tissue of the bladder (HG_MIBC). Out of those, four patients had initial and one follow up diagnosis. Our study aimed to identify differential expression genes between the three subgroups. Samples were divided up into batches of 14 and 22 samples which were sequenced independently.Read count derived from a panel of 1,410 genes was subjected to an analysis with DESeq2. We performed principal component analysis (PCA), as well as hierarchical and non hierarchical clustering. For hierarchical clustering we attempted to identify the most differentially expressed genes in both batches of samples. No specific clusters were observed for LG, HG and HG_MIBC during PCA. Differentially expressed genes could be identified during hierarchical clustering of the LG group against HG_MIBC. Of those BMP7, GATA2 and OLR1 were identified with at least a 2fold change and a $P < 0.05$ (adjusted for multiple testing). We did not identify genes differentially expressed between HG and HG_MIBC or LG.While differentiation between early and late stages of urothelial carcinomas seem possible, the limited number of targets in the gene panel or the heterogeneity of the tumor type did not allow for identification of stages in between.

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P12.198.B FISH UroVysion test and mRNA-based urine test in detection of urothelial carcinoma

Andreja Zagorac, *Ivan Perić, Niko Kavčič, Nadja Kokalj Vokač*

University Medical Centre Maribor, Maribor, Slovenia.

Introduction: Regular cytologies, cystoscopies and upper urinary tract imaging are still the gold standard for early diagnosis and monitoring of urothelial cancer (UC). A variety of commercially available urinary molecular markers have been introduced for detecting and monitoring UC. We compared two tests: FISH test UroVysion Bladder Cancer Kit routinely used in our laboratory and mRNA-based urinary marker test Xpert®Bladder Cancer Detection.

Material and Methods: Voided urine samples of 204 patients with hematuria or monitoring for tumor recurrence in patients previously diagnosed with UC, after negative cystoscopy were collected. Urine samples were analyzed using Xpert Bladder Cancer detection test which measures the levels of five target mRNAs (ABL1, CRH, IGF2, UPK1B, ANXA10) by RT-PCR and UroVysion Bladder Cancer Kit which is designed to detect aneuploidy for chromosomes 3, 7, 17 and loss of the 9p21 locus by FISH.

Results: 20 malignant tumors were detected: 12 bladder cancers, 6 ureter cancers and 2 renal pelvis cancers. 13 tumors were detected with both methods, one was missed with both and 6 were detected either with FISH or Xpert. FISH test had an overall sensitivity of 78%, a specificity of 93%, a negative predictive value of 96% and Xpert test had an overall sensitivity of 90%, a specificity of 85% and a negative predictive value of 98%. For 29% samples we didn't get the FISH result.

Conclusions: Both tests had high sensitivity, specificity and negative predictive value. Both methods represent a promising new tool in the management of urothelial carcinoma.

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P12.199.C Targeted sequencing of uterine lavage fluid for early detection of gynecologic cancer

Ieva Vaicekauskaite[1,2], *Diana Zilovic*[1,2], *Ruta Ciurliene*[1], *Rasa Sabaliauskaite*[1], *Sonata Jarmalaite*[1]

[1]*National Cancer Institute, Vilnius, Lithuania,* [2]*Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania.*

Introduction: Endometrial cancer (EC) is the most common gynecological malignancy worldwide, while ovarian cancer (OC) is the deadliest. The high incidence and mortality rates are attributed to the lack of effective screening techniques. Uterine

lavage is a non-invasive technique sampling cells shed into uterine cavity by EC and OC. Mutations in uterine lavage can be detected by sequencing and thus could provide an alternative to invasive biopsies unsuitable for resampling and patient monitoring. The aim of this study was to screen uterine lavage fluid and tissue samples from Lithuanian OC and EC patients for mutations related to gynecological cancer and to determine their associations with clinical features.

Materials and Methods: DNA from 51 uterine lavage and 35 tissue samples from 54 patients (32 OC, 11 EC and 11 patients with benign conditions) were analyzed by targeted NGS using Ion AmpliSeq™ On-Demand Panel targeting 10 genes commonly associated with OC and EC.

Results: Using targeted NGS we were able to detect 52 pathogenic and 38 uncertain significance SNPs in 74% (40/54) patients. 54% (28/54) SNPs were detected in 84% (27/32) both tissue and uterine lavage sample pairs. In high grade serous OC patients, the most commonly mutated genes were *BRCA1* and *TP53*, while in early stage non-serous OC and EC patients *PIK3CA, PTEN, KRAS and ARID1A* mutations were the most common.

Conclusions: our findings suggest that targeted sequencing of uterine lavage samples could be a useful non-invasive technique for gynecological cancer patient screening and molecular profiling.

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P12.200.D Novel mutations and genotype-phenotype correlations in Slovenian Von Hippel-Lindau (VHL) disease patients

Damjan Glavac[1], *Metka Ravnik-Glavač*[2], *Marija Pfeifer*[3], *Dušan Flisar*[4], *Katrina Novak Andrejčič*[5], *Marko Hawlina*[6]

[1]*Department of Molecular Genetics, Institute of Pathology, Faculty of Medicine, Ljubljana, Slovenia,* [2]*Institute of Biochemistry, Faculty of Medicine, Ljubljana, Slovenia,* [3]*Faculty of Medicine, Ljubljana, Slovenia,* [4]*Department of Neurology, University Medical Centre, Ljubljana, Slovenia,* [5]*Eye Hospital, University Medical Centre, Ljubljana, Slovenia,* [6]*Eye Hospital, University Medical Centre, Ljubljana, Slovenia.*

Introduction: Von Hippel-Lindau (VHL) disease (MIM no. 199300) is an autosomal dominant familial cancer syndrome with the estimated incidence 3/100 000. The most common phenotypes are retinal and cerebellar haemangioblastomas, renal cell carcinoma, phaeochromocytoma and renal, pancreatic and epididymal cysts. In our study we have focused to specific and novel VHL mutations mostly related to retinal and cerebellar haemangioblastomas manifestation in Slovenian population.

Materials and Methods: Retrospective analysis of Slovenian VHL families included 70 patients and family members. Sanger sequencing and MPLA (Multiplex ligation-dependent probe amplification) methods were used for mutation identification in the VHL gene.

Results: Two novel missense mutations p.Leu153Pro, p. Ile151Asn and one new frameshift mutation p.Arg176fs in the VHL gene were identified. Also known low penetrance p.Glu70Lys mutation was identified in three of our VHL patients. The most common ocular finding comprised retinal haemangioblastomas (13 eyes), optic disc haemangioblastoma (1 eye), optic nerve haemangioblastoma (2 eyes) and optic disc atrophy following papilledema.

Conclusions: Two novel missense mutations p.Leu153Pro, p. Ile151Asn and p.Glu70Lys mutations are related exclusively to retinal and cerebellar haemangioblastomas, while p.Arg176fs mutation phenotype includes also phaeochromocytoma. p. Glu70Lys mutation, in most populations considered as low penetrance mutation, in our population showed 100% of penetration, indeed with the very late onset in one patient who was first identified with retinal haemangioblastoma only at age 61 years. Our molecular study increases the list of known VHL mutations and contributes to a better understanding of the genotype/phenotype correlations in the VHL families. Supported by Slovenian Research Agency programme P3-0054.

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P12.201.A High rate of (epi)genetic predisposing factors and an important role for *DIS3L2* in a nationwide Wilms tumor cohort

Janna A. Hol[1], *Roland P. Kuiper*[1], *Freerk Van Dijk*[1], *Esmé Waanders*[2], *Marco J. Koudijs*[1,2], *Reno Bladergroen*[1], *Simon Van Reijmersdal*[1], *Lionel M. Morgado*[1], *Jet Bliek*[3], *Paola Lombardi*[3], *Saskia Hopman*[2], *Jarno Drost*[1,4], *Ronald R. De Krijger*[1,2], *Marry M. Van den Heuvel-Eibrink*[1], *Marjolijn C. J. Jongmans*[1,2]

[1]*Princess Máxima Center for Pediatric Oncology, Utrecht, Netherlands,* [2]*University Medical Center Utrecht / Wilhelmina Children's Hospital, Utrecht, Netherlands,* [3]*Amsterdam UMC, University of Amsterdam, Amsterdam, Netherlands,* [4]*Oncode Institute, Utrecht, Netherlands.*

Background: Wilms tumor (WT) is the most common childhood renal tumor, associated with (epi)genetic predisposing factors including Beckwith-Wiedemann Spectrum (BWSp) and *WT1*-related syndromes. In this study, we aimed to determine the prevalence of predisposing factors in relation to phenotypic findings, and to identify novel WT predisposition genes.

Methods: Phenotypic data and diagnostic test results were collected for all children diagnosed with WT in the Netherlands (2015-2020). Comprehensive BWSp testing was performed, followed by germline (trio-) whole exome sequencing (WES).

Results: 126 patients were identified, including one familial WT. (Epi)genetic predisposing factors were present in 42/126 patients (33.3%). Heterozygous *DIS3L2* variants were identified as a novel predisposing factor in five patients (4.0%), with a second somatic hit in 4/4 (100%) tumors tested. Twenty patients (15.9%) were diagnosed with BWSp, including patients with a molecular diagnosis in blood-derived DNA (N = 8), normal kidney tissue-derived DNA with at least one additional feature of BWSp (N = 8), or solely a clinical diagnosis of classical Beckwith-Wiedemann syndrome (N = 4). Four patients without additional BWSp features harbored 11p15 methylation defects in normal kidney tissue. Remaining findings included *WT1*-related syndromes (N = 10, 7.9%), Fanconi anemia (N = 1), *REST* (N = 1) and *NF1* (N = 1) mutations. Candidate WT predisposition genes were identified which require validation in larger cohorts.

Conclusions: (Epi)genetic WT predisposing factors, including mosaic 11p15 aberrations, were present in at least 33.3% of patients with WT in this national cohort, with an important role for constitutional heterozygous *DIS3L2* variants. Based on these results, we encourage standard genetic testing after counseling by a clinical geneticist.

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P13 Genome Variation and Architecture

P13.002.C A multivariate analysis identifies genetic loci associated with atherosclerotic plaque composition and cardiovascular disease trajectory

Kai Cui[1], *Joost Mekke*[2], *Saskia Haitjema*[2], *Gerard Pasterkamp*[3], *Folkert W. Asselbergs*[1], *Michal Mokry*[3], *S W. van der Laan*[3]

[1]*Department of Cardiology, Division heart and Lungs, UMC Utrecht, Utrecht, Netherlands,* [2]*Department of Vascular Surgery, University Medical Centre Utrecht, Utrecht University, Utrecht, Netherlands,* [3]*Central Diagnostics Laboratory, Division Laboratories Pharmacy, and Biomedical Genetics, University Medical Center Utrecht, University of Utrecht, Utrecht, Netherlands.*

Background: From cross-sectional studies we have learned that the composition of atherosclerotic plaques differs between individuals, and this contributes to the inter-individual differences in susceptibility to incident coronary and cerebral events. In pathological studies, the extent and type of atherosclerosis are commonly assessed based on histological plaque characteristics that are linked to plaque rupture and erosion. A better understanding of the biology underlying variability in plaque composition will provide insights into the progression of cardiovascular diseases.

Objectives: We investigated the genetics of the plaque through multivariate and integrative genome-wide analyses (GWAS) of individual plaque characteristics.

Methods: We included carotid endarterectomy patients from the Athero-Express Biobank Study (n = 2,124) with high-density imputed data and extensive histochemical plaque phenotyping available. We used slideToolKit to quantify the number of endothelial cells, macrophages, and smooth muscle cells (SMCs), and manually assessed the number of intraplaque vessels, the amount of collagen and calcification, the atheroma size, and the presence of plaque hemorrhage. We ran GWAS on all traits correcting for age, sex, array used, and genetic ancestry.

Results: We identified 3 loci that significantly associate with CD68+ macrophages and ACTA2+ SMCs, $p < 5x10^{-8}$. Statistical fine-mapping revealed 9 variants in the 95% credible set and functional annotation linked these to genes associated with malignant neoplasms, circulating cholesterol, and transmembrane proteins, suggesting an effect on cellular proliferation and cholesterol metabolism.

Conclusions: We provide evidence for 3 loci that modulate plaque composition through macrophages and smooth muscle cell plaque proliferation and cell-cell interactions.

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P13.003.D Association of genomic factors for oral health in the cohort of the Lithuanian Chernobyl catastrophe liquidators

Aušra Matulevičienė, *Gabrielė Žukauskaitė, Ingrida Domarkienė, Vaidutis Kučinskas, Laima Ambrozaitytė*

Department of Human and Medical Genetics, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, Vilnius, Lithuania.

Introduction: Ionizing radiation (IR) is one of the most significant environmental factors, affecting human health. It has a severe impact not only at high but at mild and/or persistent doses of irradiation. ADAPT (Adaptive genetic mechanisms - a comprehensive study of whole genome variation in the group of the Lithuanian Chernobyl catastrophe liquidators (LCCLs)) research project analyses the unique group of (93) LCCLs, who survived for more than 30 years after the accident. In addition to other multifactorial diseases, affecting cardiovascular (75%), skeletal and connective tissue (71%), gastrointestinal (70%) and other systems, almost half of them (48%) have oral health problems (OHP), such as periodontitis and tooth loss. In this study, we aim to identify genes that are related to the ethiopathogenesis of OHP in the LCCLs.

Materials and Methods: DNA from 93 LCCLs was extracted from venous blood and microarray genotyping was performed. These individuals were divided by their health status into case (with OHP) and control groups (without OHP). Association analysis was performed using SNPs (909) of the genes involved in tooth health (59 genes). A *chi-square* test was performed using *PLINK*.

Results: This study identified new associations between (*FGF1, FGF2, FGF7* and *BMP2*) genes to high risk of dental health problems: rs308388 ($p = 0.003807$; $OR = 2.553$), rs34022 ($p = 0.005236$; $OR = 2.389$), rs1696244 ($p = 0.003016$; $OR = 2.436$), rs3178250 ($p = 0.004969$; $OR = 3.493$).

Conclusions. Our preliminary results identified four new associations that could underlie the pathogenic IR effects on dental health. This project has received funding from the Research Council of Lithuania (LMTLT), agreement No. S-MIP-20-35.

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P13.004.A Looking back on copy gains: a retrospective review of clinical relevance and structural mechanisms

Sónia Custódio, *Rosário Silveira-Santos, Raquel Rodrigues, Eva Rolo, Juliette Dupont, Patrícia Dias, Oana Moldovan, Catarina Machado, Márcia Rodrigues, Mariana Soeiro Sá, André Travessa, João Rodrigues Alves, Raquel Gouveia Silva, Ana Berta Sousa, Ana Sousa*

Serviço de Genética Médica, Departamento de Pediatria, Hospital de Santa Maria - Centro Hospitalar Universitário Lisboa Norte, Lisboa, Portugal.

Introduction: Assessing the clinical significance of copy gains is often challenging for genetic laboratories. While copy loss interpretation is usually based on haploinsufficiency, copy gain effects are much harder to predict, bringing uncertainty to genetic reports and distress to clinical counseling.

Methods: We performed a retrospective analysis of 112 copy gains identified by aCGH (180K CGX-HD, PerkinElmer) in 98 postnatal cases reported from 2015 to 2020. Forty CNVs were further characterized by FISH or karyotype.

Results/Conclusions: 43%(48/112) were classified as pathogenic and include CNVs integrating chromosomal rearrangements [31%(15/48)]; known susceptibility loci of incomplete penetrance [27%(13/48)], syndromic microduplication regions [23%(11/48)], size >8Mb [17%(8/48)], and presence of dosage-sensitive genes [2%(1/48)]. Parental studies were performed for 26 CNVs: 12(46%) were de novo, 10(39%) were inherited, and 4(15%) originated from balanced parental rearrangements. 57%(64/112) were classified as variants of uncertain clinical significance. The latter were either regions with no clear disease association [70%(45/64)], or susceptibility loci with low penetrance (cut-off set at 20%)[30% (19/64)]. Inheritance was assessed in 34 CNVs: 28(82%) were inherited and 6(18%) were de novo. All 13 low penetrance CNVs with parental studies were inherited, these brought significant uncertainty to reports. Apart from the latter and excluding CNVs on the X chromosome, 12 were inherited from healthy parents, favoring benignity and lessening the burden of uncertainty. Of the

40 cases with structural information, the majority was in tandem [25(62%)], but chromosome rearrangements (insertions, derivative chromosomes and supernumerary marker chromosomes) were identified in 15(38%), stressing the importance of cytogenetic studies for CNVs >1Mb.

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P13.006.C High throughput analysis of disease repeat expansions and contractions by optical mapping

Dong Zhang, *Ernest Lam, Jian Wang, Tom Wang, Andy Pang, Henry Sadowski, Alex Hastie, Mark Oldakowski*

bionano genomics, San Diego, CA, USA.

Expansions and contractions of unstable repeats are associated with a range of degenerative disorders such as myotonic dystrophy and facioscapulohumeral muscular dystrophy (FSHD). These disorders most often involve trinucleotide repeats but have been associated with other types of repeat arrays. This can impact the age of onset in both the current and successive generations. The phenotype severity is often correlated with the amount of pathogenic expansion or contraction. Thus, accurate sizing of the repeats is crucial. Southern blotting is the gold standard for analyzing pathogenic repeats. Polymerase chain reaction (PCR) is used as well, but the polymerase is sometimes not able to traverse through long repeats. Sequencing-based methods are hampered by limitations in read lengths and the repetitive and polymorphic nature of these regions. Optical genome mapping with the Bionano Saphyr platform offers several advantages. With ultra-high molecular weight DNA in nanochannels, one can span and size large repeat arrays. We analyzed the FMR1 repeat relevant to Fragile X syndrome using Coriell cell lines. We observed the expected expansion alleles in the Coriell cell lines, with sizes consistent with annotation, with the largest expansion being almost 1000 copies. The control samples had repeats below the pathogenic cutoff. We also analyzed the DZ4Z repeat on chromosome 4 for FSHD. Bionano offers sample preparation, DNA imaging and genomic data analysis technologies combined into one streamlined workflow that enables high-throughput genome-wide analysis of tandem repeat regions of interest. Together, these components allow for efficient analysis of diseases associated with repeat expansion and contraction.

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P13.007.D Long-read single molecule sequencing to study the effect of CNV on transcript length of the bacteria-binding mucosal glycoprotein DMBT1

Adel Alharbi[1], *Nongfei Sheng*[2], *Nicklas Strömberg*[2], *Edward Hollox*[1]

[1]*University of Leicester, Leicester, United Kingdom,* [2]*Umeå University, Umeå, Sweden.*

The *DMBT1* gene codes for the 340kDa-DMBT1 glycoprotein, predominantly expressed in saliva and at mucosal surfaces, and binds to a wide variety of pathogens through tandemly-arranged scavenger receptor cysteine-rich (SRCR) domains. *DMBT1* shows extensive multi-allelic germline copy number variation (CNV) across all populations, with the tandemly-repeated CNV leading to different alleles with between (7-21) SRCR domains. These alleles alter the binding of DMBT1 to bacteria. This study aimed to investigate the effect of different numbers of SRCR-repeats variation on transcript length and protein length. We used a PCR-based method called the paralogue ratio test and long-range PCR to precisely genotype the SRCR-repeat number in a human lung cell line (H292). Using Oxford Nanopore sequencing, we also sequenced cDNA the same cell line, with the aim of revealing the length and nature of DMBT1 transcripts. Four full-length DMBT1 transcripts sequenced using a single sequencing read (6.7kb) showed a SRCR-domain number consistent with the genotype. Finally, we compared DMBT1 protein size from saliva, determined using Western blot, with DNA-based genotype of SRCR-domain number in a small cohort of healthy individuals to explore the relationship of the CNV encoding the SRCR-domain number to protein length. We found the genotype variation correlated with DMBT1 protein size variation, with small DMBT1 proteins correlated with fewer SRCR exons. Previous studies have suggested that alternative splicing or variable glycosylation affects DMBT1 transcript length and protein size. Our observations do not rule this out completely, but strongly stress the importance of genetically encoded CNV in DMBT1 protein variation.

A. Alharbi: None. **N. Sheng:** None. **N. Strömberg:** None. **E. Hollox:** None.

P13.008.A VariantAlert: a free service to notify updates in genetic variant annotations

Rossano Atzeni[1], *Matteo Massidda*[1], *Giorgio Fotia*[1], *Paolo Uva*[2,3]

[1]*Center for Advanced Studies, Research and Development in Sardinia (CRS4), Pula, Italy,* [2]*IRCCS Giannina Gaslini, Genova, Italy,* [3]*Italian Institute of Technology, Genova, Italy.*

Introduction: Variant reinterpretation based on the availability of updated annotations is part of the routine work of research laboratories: the more data is collected about a specific variant, the higher the probability to reinterpret an unclassified variant. To support the interpretation of genetic variants, we developed VariantAlert, a web-based tool to help researchers and clinicians to keep informed about changes in variant annotations extracted from multiple sources.

Materials and Methods: VariantAlert is a web application built in Django, and uses PostgreSQL as the database backend. The web server is Nginx. Travis CI performs continuous integration, automatically running the test suite whenever the codebase is changed on Github repository. VariantAlert is easy to install locally or deploy remotely through the use of the Docker platform. A Makefile allows users to easily start VarianAlert, taking care of installation, configuration and running steps.

Results: A user can submit one or more lists of variants which are daily re-annotated using external resources accessed through API, such as MyVariant.info annotation API providing links to variant annotations from gnomAD, COSMIC, ClinVar, CIViC, and many others. If a change is detected for the annotation of a variant due to the upgrade of the underlying resource the user is notified by email and updated annotations are stored on the web-site.

Conclusions: VariantAlert contributes to the interpretation of genetic variants and their classification by keeping the researchers constantly informed of the current content of multiple annotation databases (See https://variant-alert.crs4.it/)

R. Atzeni: None. **M. Massidda:** None. **G. Fotia:** None. **P. Uva:** None.

P13.009.B Large genomic imbalances and phenotype

Martine Doco-Fenzy[1,2,3], *Jean Michel Dupont*[2,4], *Chantal Missirian*[2,5], *Patrick Callier*[2,6], *Valérie Malan*[7,8], *Sylvia Redon*[2,9], *Emilie Landais*[1], *Lucas Hérissant*[1], *Paul Kuentz*[2,10], *Gaelle Vieville*[2,11], *Marie Faoucher*[2,12], *Olivier Pichon*[7,13], *Mathilde Pujalte*[2,14], *Perrine Pennamen*[7,15], *Caroline Thambo*[7,16], *Cedric Lecaignec*[17], *Boris Keren*[7,18], *Sylvie Jaillard*[2], *Jonathan Levy*[2,19], *Christine Pebrel*[2,20], *Nathalie Douet*[9], *Céline Bonnet*[21], *Mathieu Egloff*[22], *Eva Pipiras*[2,23], *Charles Coutton*[2,24], *Pascal Chambon*[25], *Dorothée Reboul*[26], *François Vialard*[2,27], *Anne -Claude Tabet*[2,19], *Nicolas Chatron*[2,28], *Caroline Schuth Bolard*[2,29], *Nicolas Gruchy*[2,30], *Vincent Gatinois*[2,31], *Damien Sanlaville*[7,28]

[1]*CHU REIMS, REIMS, France,* [2]*ACLF, Paris, France,* [3]*EA3801 SFR CAP SANTE, Reim, France,* [4]*CHRU Cochin, Paris, France,* [5]*CHRU Marseille, Marseille, France,* [6]*CHRU Dijon, Dijon, France,* [7]*Achropuce, Paris, France,* [8]*CHRU Necker, Paris, France,* [9]*CHRU Brest, Brest, France,* [10]*CHU de Besançon, Besançon, France,* [11]*CHU de Grenoble, Grenoble, France,* [12]*CHRU de Bordeaux, Bordeaux, France,* [13]*CHU de Nantes, Nantes, France,* [14]*CHU d'Amiens, Amiens, France,* [15]*CHU de Bordeaux, Bordeaux, France,* [16]*CHU Bordeaux, Bordeaux, France,* [17]*CHU Toulouse, Tououse, France,* [18]*CHU La Pité Salpétrière, Paris, France,* [19]*CHU Robert Debré, Paris, France,* [20]*CHU Clermont Ferrand, Clermont Ferrand, France,* [21]*CHRU de Nancy, Nancy, France,* [22]*CHU Poitiers, Poitiers, France,* [23]*CHU Jean Verdier, Bondy, France,* [24]*CHRU de Grenoble, Grenoble, France,* [25]*CHU Rouen, Rouen, France,* [26]*CHU Nimes, Nimes, France,* [27]*CHU de Versailles, Versailles, France,* [28]*CHU Lyon, Bron, France,* [29]*CHRU de Lyon, Lyon, France,* [30]*CHU de Caen, Caen, France,* [31]*CHRU Montpellier, Montpellier, France.*

Introduction: The NGS Genome study, improves the "limited" karyotype observations and gives keys to better understand the phenotype-genotype links. In routine analysis for ID or malformations, array-CGH technique identifies genome losses or gains, imbalances ranging from the exon scale down to that of entire chromosome. This technique has made us progress in the understanding of CNVs with their impact on TADs (topologically associated domains). The interpretation of the pathogenicity of CNVs follows guidelines based notably on size and inheritance. But today we remain faced with problems of interpreting the pathogenicity of large CNVs over 3Mb inherited from normal parents, sometimes in a clinical emergency. The purpose of our studies is to establish the composition of the regions of the genome over 3 or 5 Mb which can be in variable copy number without phenotypic consequence. This with two sub-questions: do they have common characteristics that would allow a genetic counseling in prenatal context and why do large CNV without phenotypic consequence in a parent is expressed in his offspring? **Material and methods:**to answer these questions we have collected among our networks (ACLF and Achropuces) the data of large CNVs without phenotypic consequences in order to map these changes and make a bioinformatic analysis of their characteristics. The preliminary data on 29188 MCA test show 5710 CNVs over 1Mb, among them 40,3% are between 1 and 2Mb. 16,8% between 2 and 3Mb, 7,4% between 3 and 4Mb and interestingly 15,22% over 10Mb. We report the detailed data of the cohort.

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P13.012.A Two novel deletions in the 5 Untranslated region of *GNAS* gene as a cause of pseudohypoparathyroidism type 1A

Louise-May Thibaut[1], *Arnaud Molin*[1], *Christine Francannet*[2], *Benjamin Dauriat*[3], *Andreea Apetrei*[1], *Nicolas Richard*[1]

[1]*Normandy University Hospital, Caen, France,* [2]*Clermont-Ferrand University Hospital, Clermont-Ferrand, France,* [3]*Limoges University Hospital, Limoges, France.*

The *GNAS* complex locus encodes the biallelically expressed α subunit of the stimulatory G protein (Gαs) and additional imprinted or non-coding transcripts. Pseudohypoparathyroidism type IA (PHP1A) is a rare disease caused by a decrease in the activity of Gαs, characterized by multihormone resistance and the Albright Hereditary Osteodystrophy phenotype (round facies, short stature, subcutaneous ossifications, brachydactyly, and early-onset obesity). The reason of this disorder is maternal inactivating mutations involving Gαs exons.

In this study we investigated two affected individuals, unrelated, both presenting a typical phenotype of PHP1A. A methylation-specific multiplex ligation-dependent probe amplification analysis and a targeted Next Generation Sequencing were achieved for the two siblings but were not contributive. A whole genome sequencing (WGS) analysis was performed: the library preparation (paired-end 2x150 bp) used the NEBNext DNA Library Prep Kit. The sequencing was performed using an Illumina platform and the sequences were aligned at the reference human genome GRCh38. The data was then processed using several pipelines (structural variant, variant calling, copy number variant).

The structural variant detected two novel heterozygous deletions in the 5′ Untranslated region of *GNAS* gene for each sibling, 466 bp and 1439 bp respectively. These deletions were confirmed by a PCR analysis of the genomic DNA using primers designed to amplify across both breakpoints of the mutant allele.

The WGS contributes considerably to these cases without previous diagnosis using standard tecnhiques especially on the appearance of structural variants. Developing additional strategies should be interesting so as to detect these potentially recurring deletions.

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P13.013.B A novel synonymous-predicted variant in exon 1 of *GNAS* gene results in a cryptic splice site and causes pseudohypoparathyroidism type 1A and pseudopseudohypoparathyroidism in a French family

Andreea Apetrei[1], *Arnaud Molin*[1], *Nicolas Gruchy*[1], *Manon Godin*[1], *Claire Bracquemart*[1], *Antoine Resbeut*[1], *Gaëlle Rey*[2], *Gwenaël Nadeau*[2], *Nicolas Richard*[1]

[1]*Department of Genetics, CAEN, France,* [2]*Department of Genetics, Chambéry, France.*

Introduction: Pseudohypoparathyroidism type 1A (PHP1A) and Pseudopseudohypoparathyroidism (PPHP) (Inactivating PTH/PTHrP Signaling Disorders type 2, IPPSD2) are two rare autosomal disorders caused by loss-of-function mutations in the imprinted *GNAS* gene, which encodes the α subunit of the ubiquitously-expressed G protein (Gαs).

Material and Methods: We investigated a French family including 2 patients presenting an IPPSD2 phenotype. *GNAS* exons 1-13 and intron/exon boundaries were sequenced and interpreted following the routine protocol in our molecular genetics laboratory. Two splicing-prediction algorithms were used. Quantitative reverse-transcription polymerase chain reaction was used to assess the expression of *GNAS*. Reverse transcription polymerase chain reaction using a gene-specific primer was realized in order to identify the mutant allele.

Results: We identified a synonymous *GNAS* variant NM_001077488.2:c.108C>A / p.(Val36=) present in the affected members with IPPSD2 phenotype. *In silico* splicing prediction algorithms were in favor of a deleterious effect of this variant, by creating a new donor splicing site. The *GNAS* expression studies in blood suggested haploinsufficiency and showed an alternate splice product demonstrating the unmasking of a cryptic site, leading to a 34 base pairs deletion and possibly, the creation of an unstable RNA.

Conclusions: We present the first familial case of IPPSD2 caused by a pathogenic synonymous-predicted variant in *GNAS* gene. This observation supports the increasing interest in the identification of splicing variants, particularly those predicted as synonymous, which are often systematically categorized as benign. Splice-prediction algorithms could be added to the bioinformatic pipelines for MPS data in routine analysis to improve the detection of such variations.

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P13.014.C Analysis of neurotransmitter gene expression and comparison with the receptor density in human hippocampal regions

Thomas W. Mühleisen[1,2,3], *Ling Zhao*[1], *Dominique Hilger*[1], *Bettina Burger*[3], *Andreas Forstner*[1,4], *Stefan Herms*[3,4], *Per Hoffmann*[4,3], *Karl Zilles*[1], *Katrin Amunts*[1,2], *Sven Cichon*[1,3,5], *Nicola Palomero-Gallagher*[1,2,6]

[1]*INM-1, Jülich, Germany,* [2]*Cécile and Oskar Vogt Institute for Brain Research, Medical Faculty, Heinrich Heine University Düsseldorf, Düsseldorf, Germany,* [3]*Department of Biomedicine, University Hospital Basel and University of Basel, Basel, Switzerland,* [4]*Research Platform Genomics, Institute of Human Genetics, University Hospital of Bonn, Bonn, Germany,* [5]*Institute of Medical Genetics and Pathology, University Hospital Basel, Basel, Switzerland,* [6]*Department of Psychiatry, Psychotherapy and Psychosomatics, RWTH Aachen University, Aachen, Germany.*

Introduction: The hippocampus plays a crucial role in memory and learning. We screened for differential expression of neurotransmitter receptors and their genes in the cornu ammonis (CA) and dentate gyrus (DG), to gain insight into their regional specificity. We directly compared RNA transcripts and protein densities from the same donor samples.

Methods: Seven fresh-frozen samples were obtained at autopsy. Donors (71.4 ± 15 years) were free from neurological or psychiatric diseases. RNA expression was genome-wide analysed and normalized. Receptor densities (protein expression levels) were quantified by autoradiographic analysis.

Results: We analysed four transmitter systems. Highest RNA expression both in CA and DG were found for the muscarinic cholinergic and adenosinergic systems, followed by the serotonergic and dopaminergic systems. Highest protein density showed the adenosinergic and serotonergic systems in both regions, followed by the cholinergic and dopaminergic systems. Relationship between RNA expression and protein level differed between CA and DG for different receptors. Genes and proteins of some transmitter systems were positively correlated (e.g. *ADORA1* and A1) while others were negatively correlated in either one or both regions.

Conclusions: Our findings suggest the presence of region- and receptor type-specific regulatory mechanisms between CA and DG. A deduction of receptor densities from gene expression data alone may therefore be challenging. We hypothesize that the identified differences could be associated with region-specific plasticity mechanisms in learning and memory processes. **Funding:** This project has received funding from EU's Horizon 2020 programme "Human Brain Project" under Agreements 785907 (SGA2) and 945539 (SGA3).

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P13.015.D Role of splicing regulatory elements and *in silico* tools usage in the identification of splicing altering deep intronic variants

Joanna Domènech-Vivó[1], *Alejandro Moles-Fernández*[1], *Anna Tenés*[2], *Sara Hermosa-García*[1], *Orland Diez*[1,2], *Sara Gutiérrez-Enríquez*[1]

[1]*Hereditary Cancer Genetics Group, Vall d'Hebron Institute of Oncology (VHIO), Vall d'Hebron Hospital Campus, BARCELONA, Spain,* [2]*Area of Clinical and Molecular Genetics, Vall d'Hebron Hospital Universitari, Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spain.*

Deep intronic variants can alter splicing, including intronic regions in mRNA by activating/creating splicing sites or splicing regulatory elements (SREs). However, these alterations remain underestimated. Although different computational tools separately identify variants affecting cryptic sites (SPLICEAI, MES, SSF or HSF) and SREs (ESRseq), there is no specific pipeline to assess the splicing defects induced by intronic changes. Our aim is to provide a validated *in silico* algorithm to identify them. After a bibliographic review, the spliceogenic effect of several deep intronic variants was compiled in a pilot set and used to evaluate the performance of SPLICEAI and to characterize the landscape of SREs in pseudoexons and canonical exons by ESRseq. The results permitted to generate a pipeline to prioritize variants for RNA analysis, which was validated in independent datasets. SpliceAI reached 86% sensitivity and 92% specificity with a threshold of 0.05. However, its performance was lower in a dataset of exclusively SRE creating/disrupting variants. Using ESRseq we proved that pseudoexons were significantly enriched in enhancer regulatory elements, although they were more abundant in canonical exons. The analysis of independent experimental and literature data showed that the sequential combination of both tools was able to detect 85% of variants disrupting splicing. This work provides the first validated *in silico* pipeline, combining tools considering different splicing conserved elements, to prioritize deep intronic variants for RNA analysis, which will improve the variants of unknown significance classification. Carlos III Institute funded by FEDER-a way to build Europe- [PI16/01218-PI19/01303]; AGAUR FI-DGR2020 and ERAPERMED2019-215 support.

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P13.018.C Case report of two Brazilian families with Li-Fraumeni Syndrome phenotype with a variant of uncertain significance in TP53

Deivid C. de Souza, *Gisele Santos Oliveira, Vanessa N. Kozak*

Hospital Erasto Gaertner, Curitiba, Brazil.

Introduction: Li-Fraumeni syndrome (LFS) is a genetic disorder that predisposes to a wide-spectrum of tumors (premenopausal breast cancer, soft-tissue sarcoma, osteosarcoma, central nervous system tumor, adrenocortical carcinoma) at an early age. While more than 70% of pathogenic variants in TP53 are missense variants, including the most common variant in southern Brazil (TP53:c.1010G>A, p.Arg337His), the vast majority occurs very infrequently, and thus their clinical significance is uncertain or conflicting.Case report: We herein report the cases of two unrelated families with the same genetic variant (TP53:c.718A>G, p.Ser240Gly), which has conflicting interpretations of pathogenicity in ClinVar (variant of uncertain significance vs. likely pathogenic). Both families fulfill the Chompret criteria for Li-Fraumeni Syndrome. Intriguingly, a member of one of the families had a high-grade serous ovarian cancer (malignant neoplasia not usually associated with Li-Fraumeni Syndrome) at 23 years of age.

Conclusion: Based on our analysis of these two different Brazilian families, we suggest that TP53:c.718A>G may be a clinically significant variant. Although ovarian cancers have been found to occur excessively in at least some families who have met criteria for LFS, their link to the syndrome is not definitely established. The observation of an individual with early-onset ovarian carcinoma can further contribute to our understanding of the phenotypic variability that may be caused by one variant of TP53, even within the same family.

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P13.021.B MicroRNA binding sites and their potential role in human disease

*Olga Plotnikova[1], **Alexandra Filatova**[2], Mikhail Skoblov[2]*

[1]Moscow Institute of Physics and Technology, Moscow, Russian Federation, [2]Research Centre for Medical Genetics, Moscow, Russian Federation.

Introduction: MicroRNAs play an important role in regulation of gene expression and can be associated with human disease. Despite the extensive research, bioinformatics prediction of microRNA binding sites remains a challenge. Available tools still lack accuracy and sensitivity, complicating their use for studying role of microRNA-binding sites mutations in human disease. Previously, we systematically analyzed the experimentally identified mRNA-microRNA duplex regions from available CLASH and CLIP datasets. In the present work, we use the obtained data to identify microRNA-binding sites, potentially associated with cancer and hereditary disorders.

Materials and Methods: To search for potential disease-associated microRNA-binding sites we used previously created by us "Exp-miBR annotator" tool (http://score.generesearch.ru/services/mirna/) as well as available data from COSMIC and ClinVar databases. For experimental study of microRNA-binding sites, we used psiCHECK2 luciferase system. Protein and mRNA expression level was measured using luciferase dual-assay and RT-PCR.

Results: Bioinformatic analysis revealed 148 high-confidence microRNA-binding sites in human genes belonging to the COSMIC tier1 oncogenes group. We selected four out of them for experimental validation (in *CDK6*, *CCND2*, *DEK*, *SRSF2* genes) and showed that investigated microRNA-mRNA interactions could regulate gene expression through various mechanisms. Further analysis of pathogenic variants from ClinVar identified 961 variants localized in high-confidence microRNA-binding sites and only six variants were localized in 3'UTRs. For two of them we also performed an experimental test.

Conclusions: Our previous work reported a high-confidence microRNA-mRNA interactions allowed us to identify microRNA-binding sites, mutations in which could potentially play role in the development of human cancer and hereditary disorders.

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P13.022.C Risk of mitochondrial deletions is affected by the global secondary structure of the mitochondrial genome

Victor Shamanskiy[1], *Alina A. Mikhailova[2,1], Kristina Ushakova[1], Alina G. Mikhailova[1,3], Sergei Oreshkov[1], Dmitry Knorre[4,5], Evgenii O. Tretiakov[6], Marianna Zazhytska[7], Samuel W. Lukowski[8], Chia-Wei Liou[9], Tsu-Kung Lin[9], Wolfram S. Kunz[10,11], Alexandre Reymond[12], Ilia Mazunin[13,14], Georgii A. Bazykin[13,15], Konstantin Gunbin[1,16], Jacques Fellay[17], Masashi Tanaka[18,19,20], Konstantin Khrapko[21], Konstantin Popadin[1,12,17,22]*

[1]Center for Mitochondrial Functional Genomics, Immanuel Kant Baltic Federal University, Kaliningrad, Russian Federation, [2]Institute for Evolution and Biodiversity, University of Münster, Münster, Germany, [3]Vavilov Institute of General Genetics RAS, Moscow, Russian Federation, [4]Laboratory of Systems Biology and Computational Genetics, Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, Russian Federation, [5]Institute of Molecular Medicine, Sechenov First Moscow State Medical University, Moscow, Russian Federation, [6]Department of Molecular Neurosciences, Center for Brain Research, Medical University of Vienna, Vienna, Austria, [7]Department of Biochemistry and Molecular Biophysics, Mortimer B. Zuckerman Mind Brain and Behavior Institute, Columbia University, New York, NY, USA, [8]Institute for Molecular Bioscience, University of Queensland, Brisbane, Australia, [9]Neurology, Kaohsiung Chang-Gung Memorial Department of Hospital and Chang-Gung University, Kaohsiung, Taiwan, [10]Division of Neurochemistry, Department of Experimental Epileptology and Cognition Research, University Bonn, Bonn, Germany, [11]Department of Epileptology, University Hospital of Bonn, Bonn, Germany, [12]Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland, [13]Center of Life Sciences, Skolkovo Institute of Science and Technology, Skolkovo, Russian Federation, [14]Department of Genomic Medicine, Fomin Women's Health Clinic, Moscow, Russian Federation, [15]Sector of Molecular Evolution, Institute for Information Transmission Problems (Kharkevich Institute) of the Russian Academy of Sciences, Moscow, Russian Federation, [16]Institute of Molecular and Cellular Biology SB RAS, Novosibirsk, Russian Federation, [17]School of Life Sciences, Ecole Polytechnique Federale de Lausanne, Lausanne, Switzerland, [18]Department for Health and Longevity Research, National Institutes of Biomedical Innovation, Health and Nutrition, Tokyo, Japan, [19]Department of Neurology, Juntendo University Graduate School of Medicine, Tokyo, Japan, [20]Department of Clinical Laboratory, IMS Miyoshi General Hospital, Saitama, Japan, [21]Biology Department, Northeastern University, Boston, MA, USA, [22]Department of Life Sciences, Swiss Institute of Bioinformatics, Lausanne, Switzerland.

Ageing is often associated with clonal expansion of somatic mitochondrial deletions, while their origin is still poorly known. Deletions are often flanked by direct nucleotide repeats, however, repeats solely do not provide an exhaustive explanation of deletion distribution. Here, we hypothesized that repeats have higher chances to be realized into deletions in case of their spatial proximity. Analyzing the distribution of human deletions we observed a hot spot (6-9kb and 13-16kb), which is not explained by direct repeats and might be driven by close contacts of these two regions during mtDNA replication. Using several in silico

approaches we reconstructed the secondary structure of the major arc and proposed that it is organized as a large-scale hairpin-like loop with a center close to 11 kb and stem between 6-9 kb and 13-16 kb. mtDNA Hi-C data of healthy and COVID-19 patient samples also demonstrated a high-density region in the expected contact zone. In our final model, we demonstrated that repeats within the contact zone are 3-times more mutagenic as compared to repeats outside the contact zone, which clarifies also well known increased mutagenicity of the common repeat (8470-8482 bp and 13447-13459 bp). The proposed topological model improves our understanding of the mechanisms of deletion formation in the human mitochondrial genome and opens a possibility to predict deletion burden in different human haplogroups and mammalian species.

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P13.023.D Mobile Element Insertion: Mild haemophilia B caused by *HNRNPC* processed pseudogene inserted in the *F9*

*Amy Dericquebourg[1,2], **Nicolas Chatron**[3,4], Mathilde Frétigny[1], Claude Négrier[1,2], Christine Vinciguerra[1,2], Yohann Jourdy[1,2]*

[1]Service d'hématologie Biologique, Centre de Biologie et Pathologie Est, Hospices Civils de Lyon, Lyon, France, [2]EA 4609 Hémostase et cancer, Université Claude Bernard Lyon 1, Lyon, France, [3]Genetics Department, Lyon University Hospital, Lyon, France, [4]NeuroMyoGène Institute, University of Lyon, Claude Bernard University Lyon 1, Lyon, France.

The conventional genetic exon-focused approaches fails in about 1% of haemophilia B patients. We hypothesized that deep intronic variations could be pathogenic and this motivated our strategy for whole-*F9* sequencing by targeted short-read paired-end sequencing. Herein, we report a genetically unresolved mild haemophilia B patient (FIX:C = 46 IU.dL^{-1}) harboring a pathogenic retrotransposition of a mobile element. In *F9* intron 6 (c.727 +1853_1854ins), we identified the insertion of a 1.377 kb processed pseudogene (retrocopy) of *HNRPNC* transcript (NM_004500.6) lacking the end of the 3'UTR, in opposite orientation to the *F9*. A single other variant was discarded after minigene assay. The sequence of the insertion showed the hallmarks of a target-primed reverse transcription event: (i) poly(A) tail, (ii) target site duplication, and (iii) a consensus LINE-1 endonuclease cleavage site. Formal confirmation that this insertion leads to an abnormal *F9* splicing is ongoing with a minigene assay. This observation highlights the efficiency of our whole-gene approach to detect intronic structural variants and solve unexplained haemophilia B. While the rate of such retrotransposition is estimated to be around 1/6200 meiosis, this is only the second observation of a monogenic disease caused by a processed pseudogene insertion. For other diseases, we anticipate that structural variant calling on whole genome sequencing data will reveal more cases and contribute to the progressive reduction of the unsolved cases. We believe that our observation can help to raise awareness around these rare events.

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P13.024.A Multisite de novo mutations after paternal exposure to ionizing radiation

***Fabian Brand**[1], Manuel Holtgrewe[2], Leonie Weinhold[3], Alexej Knaus[1], Matthias Schmid[3], Dieter Beule[2], Peter Krawitz[1]*

[1]Institut for genomics statistics and bioinformatics, Bonn, Germany, [2]Berlin Institute of Health, Berlin, Germany, [3]Institut für Medizinische Biometrie, Informatik und Epidemiologie, Bonn, Germany.

In our ongoing study we evaluate the effects of ionizing radiation on the offspring of exposed soldiers.

We sequenced the whole genome of 270 individuals from 76 families to an average coverage of 30X on an Illumina NovaSeq. Eighteen offspring of twelve families have earlier been sequenced on a HiSeq X, three of which have now been resequenced. Our control cohort consists of 1275 families with no known exposure to irradiation which have been sequenced on HiSeq devices. We found that A>C and T>G transversions are enriched in NovaSeq data.

Our focus lies on specific mutational patterns such as MSDNs (at least two de novo mutations within 20bp) which are suspected to have a causal relationship with ionizing radiation with high linear energy transfer.

After accounting for age and sequencing platform, we found no significant difference in the mean number of de novo mutations (DNMs). We found on average 5.4 MSDNs/offspring in the case cohort and 3.9 MSDNs/offspring in the control cohort. We detected 43% more MSDNs per DNM in the case cohort ($p < 0.00001$). The number of mutations in MSDN clusters is increased 33% on average ($p = 0.018$) in the offspring of radar soldiers.

To validate our results, all complex de novo variants will be resequenced using long read techniques. These reads are used to assert the paternal origin of all MSDNs. To correlate our findings with the amount of ionizing radiation each radar soldier was subjected to during his service, we aim to incorporate retrospective dosage estimations into the analysis.

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P13.025.B Role of hypomorphic variants in variable expressivity of Noonan syndrome

***Viviana Tritto**[1], Eleonora Mangano[2], Maria T. Bonati[3], Paola Bettinaglio[1], Cristina Battaglia[1,2], Roberta Bordoni[2], Paola Riva[1]*

[1]Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, Milano, Italy, [2]Institute of Biomedical Technologies, Consiglio Nazionale delle Ricerche, Segrate (Milano), Italy, [3]Medical Genetics Unit, IRCCS Istituto Auxologico Italiano, Milano, Italy.

Introduction: Noonan syndrome (NS) is an autosomal dominant multisystem disorder, caused by mutations in RAS pathway's genes. It's characterized by a variable expressivity of specific clinical signs including craniofacial anomalies, congenital heart defects, and neurocognitive delay. Interestingly, for 20-30% of patients is not possible to provide molecular diagnosis, suggesting that different genes or mechanisms are involved in NS pathogenesis.

Materials and Methods: We studied selected variants from WES analysis of four NS patients negative to the conventional NS

mutation screening, through eVai Variant Interpretation platform (enGenome). Patients showed a digenic or compound heterozygous inheritance of RAS pathway hypomorphic variants, singularly present in healthy parents (PMID: 32514133).

Results: Five heterozygous missense mutations candidate as genetic modifiers passed our filtering steps including variants with MAF less than 0.05 and eVai pathogenicity score at least 3.5, associated with clinical conditions sharing at least 4 Human Phenotype Ontology terms with the patient. A patient showed two variants, one in CACNA1G, a gene related to Spinocerebellar ataxia with autosomal dominant inheritance, and the other one in KDM5B, a gene associated with autosomal recessive mental retardation. In each of the other patients, only one potential pathogenic variant was found. The mutated genes were PC, related to Leigh syndrome inherited as an autosomal recessive trait, SMAD4, with autosomal dominant inheritance in Myhre syndrome, and DDR2, related to Spondylometaphyseal dysplasia caused by recessive mutations.

Conclusions: These findings suggest possible modifier genes to be implicated in NS variable expressivity and phenotype severity, providing new insights in the pathogenesis.

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P13.026.C Are copy-number gains in 17p11.2 not involving *RAI1* still Potocki-Lupski Syndrome?

Christopher M. Grochowski[1], *Lorraine Potocki*[1], *Anna Lindstrand*[2], *Claudia M. B. Carvalho*[3], *James R. Lupski*[1]

[1]*Baylor College of Medicine, Houston, TX, USA,* [2]*Karolinska Institutet, Stockholm, Sweden,* [3]*Pacific Northwest Research Institute, Seattle, WA, USA.*

Potocki-Lupski syndrome (PTLS) (MIM: 610883) is a microduplication disorder caused by copy-number gains spanning the dosage-sensitive gene *RAI1*. Clinically, PTLS is defined by a spectrum of developmental delay/intellectual disability (DD/ID), infantile hypotonia and congenital anomalies. The majority of PTLS cases (64%) are caused by non-allelic homologous recombination (NAHR) between repeat gene clusters on 17p11.2, resulting in a recurrent ~3.6 Mb duplication. Interestingly, patients with copy-number gains in 17p11.2 not including *RAI1* and a DD phenotype often receive a presumed diagnosis. To date, we have ascertained 12 cases with copy-number gains in 17p11.2 not involving *RAI1* with a broad phenotype of DD/ID and performed high-resolution arrayCGH as well as whole-genome sequencing (WGS) on a subset of samples. Genomic complexities identified in this cohort include DUP-NML-DUP, DEL-DUP as well as marker chromosomes. Three cases appear to involve an identically overlapping copy number gain (including a duplication, a triplication and a 6x amplification) with one of the breakpoints mapping to the structurally polymorphic cancer associated isodicentric 17q breakpoint cluster. Further analyses in this region will help to delineate if this phenotype is i) associated with the 17p11.2 copy-number gain, ii) represents a milder form of the disease and/or iii) was due to pathogenic variation located elsewhere in the genome. Patients with atypical presentations of the normal PTLS genotype or phenotype present a chance to better delineate the spectrum of the disease. Furthermore, such gains may illuminate SV mutagenesis mechanism(s) and provide insight into potential PTLS contributing genes other than the 'driver *RAI1* gene'.

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P13.027.D Housekeeping gene and protein expression changes in CCD1079Sk cell line during passages

Omer Faruk Duzenli[1,2,3], *Emrah Yucesan*[4], *Beyza Goncu*[1,5]

[1]*Bezmialem Vakif University, Experimental Research Center, Istanbul, Turkey,* [2]*Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Genetics, Istanbul, Turkey,* [3]*Istanbul University, Graduate School of Health Sciences, Istanbul, Turkey,* [4]*Bezmialem Vakif University, Faculty of Medicine, Department of Medical Biology, Istanbul, Turkey,* [5]*Bezmialem Vakif University, Vocational School of Health Service, Department of Medical Services and Techniques, Istanbul, Turkey.*

Introduction: The genes that are commonly expressed in different tissue types are called housekeeping genes. The expression levels of certain genes or proteins may exhibit different or similar patterns regardless of the tissue type. The housekeeping genes and proteins are often used during the normalization of the mRNA and protein expressions. Herein, our aim is to define the most stable reference gene and protein for a healthy human fibroblast cell line which may be used as a candidate for functional studies.

Materials and Methods: In this study, we used a finite fibroblast cell line derived from healthy human skin (CCD1079Sk(ATCC®CRL-2097™)).The expression levels of four commonly used reference genes and proteins including *ACTB* (β-actin), *GAPDH*, *RPLP0*, and *SDHA* expression were examined. The study was performed by collecting samples in each passage between 25 and until senescence occurs at passage 55.The ct values were obtained by using qRT-PCR. Protein lysates were used for Western Blot. The stability of mRNA and protein expression were evaluated by using RefFinder.

Results: GAPDH was the most stable reference gene and protein. The least stable reference gene and protein was found RPLP0. Particularly, RPLP0 protein isoforms were detected and isoform-1 expression significantly decreased by passages.

Conclusions: Collectively, we strongly suggest that *GAPDH* will be the most suitable and stable reference gene and protein for the CCD1079Sk. The *RPLP0* gene was found unstable during each passage and using the *RPLP0* gene as a reference may be misleading. Funding: This project was supported Bezmialem Vakif University, Scientific Research Committee(No:20200917)

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P13.029.B Ring chromosome 22 in patients with 22q13 duplication, 22q13 interstitial deletion, and 22q13 terminal deletion

Anthony Nemr[1], *Tony Yammine*[1], *Rita Esber*[1], *Rita BouChedid*[1], *Melissa Daou*[1], *Georges Hilal*[1], *Chantal Farra*[1,2]

[1]*Medical Genetics Unit, Faculty of Medicine, Saint-Joseph University, Beirut, Lebanon,* [2]*Medical Genetics Department, Hotel-Dieu de France University Hospital, Beirut, Lebanon.*

Introduction: Ring chromosomes result from telomeric deletion and/or dysfunction in the short and long chromosomal arms followed by fusion of terminal ends. Numerous 22q13.3 terminal deletion cases presenting with a ring chromosome 22 have been reported. 22q13.3 chromosomal region is, furthermore, reportedly involved in few cases of interstitial deletion beyond *SHANK3* gene and rare cases of duplication.

Materials and methods: Three unrelated patients with a constitutional ring chromosome 22 karyotype were recruited upon individual or parental informed consent for further aCGH analysis and clinical data collection.

Results: In patient 1, a 22q13.32q33 duplication encompassing *SHANK3* gene was identified. The patient presented with dysmorphic features, neonatal hypotonia, severe neurodevelopmental and speech delay, as well as, Lennox-Gastaut seizures, scoliosis, hip dysplasia, and joint hypermobility. Patient 2 showed mosaic interstitial deletion of the 22q13.31q33 region, excluding *SHANK3* gene. The deleted region encompassed *UPK3A*, *FBLN1*, *ATXN10*, *WNT7B*, and *CELSR1* genes with partial involvement of *PARVB* gene. The patient presented with neurodevelopmental and speech delay, neonatal hypotonia, growth delay, and ureter anomaly. Patient 3 showed a terminal deletion of 22q13.33 region, including the gene *SHANK3*. He presented with bilateral congenital glaucoma, microcephaly, right hand angioma, and right foot syndactyly.

Conclusions: Ring chromosome 22 along with terminal duplications and interstitial deletions have not been previously reported. The cases presented here emphasize the wide range of clinical manifestations of this chromosomal anomaly while uncovering variable underlying gene rearrangements and involvements.

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P13.030.C Psychoemotional stress induces the changes in the content of satellite III (1q12) and telomere repeats in human leukocyte DNA

*Svetlana V. Kostyuk[1], **Pavel E. Umriukhin**[1,2], Elizaveta S. Ershova[1], Anton D. Filev[1], Vera A. Michailova[1], Andrey V. Martynov[1], Roman V. Veiko[1], Natalia N. Veiko[1]*

[1]Research Centre for Medical Genetics, Moscow, Russian Federation, [2]I.M. Sechenov First Moscow State Medical University, Moscow, Russian Federation.

Introduction: Previously, it was shown that CNVs of human satellite III (1q12) fragment (f-SatIII) and telomere repeat (TR) reflect human cells response to oxidative stress. The major research question: What are the f-SatIII and TR CNVs in human leukocyte as a function of psychoemotional stress.

Materials and Methods: We chose a model of psychoemotional stress experienced by the second year medical students during the exams. Blood samples were taken in a stressful conditions (preparation for exams and exams) and in a control nonstressful period. Biotinylated DNA-probes were used for f-SatIII, rDNA and TR quantitation in leukocyte DNA by the non-radioactive quantitative hybridization for seventeen medical students. Flow cytometry analysis was used for lymphocytes' oxidative stress markers (NOX4, 8-oxodG and γH2AX) detection. Involved in the DNA repair and lymphocyte death proteins levels were also determined.

Results: Oxidative stress markers (NOX4, 8-oxodG and γH2AX) increase significantly in students' lymphocytes during psychoemotional stress. BAX1, BCL2, p53, LC3, p65 (NF-kB), BRCA1, NRF2, MDM2, RAD50 and MRE11A proteins levels in the students' lymphocytes increased under stress. F-SatIII and TR contents significantly change in DNA isolated from blood leukocytes against the background of stable rDNA content. After holidays, f-SatIII content in DNA decreases, and the TR content increases.

Conclusions: Stress in students during exams induces oxidative stress and significant f-SatIII and TR content fluctuations in DNA against stable ribosomal repeat content background. A hypothesis is proposed explaining quantitative f-SatIII and TR polymorphism under stress. Study was supported by RFBR project №19-34-90072 and Russian Science Foundation (№18-15-00437).

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P13.031.D Rare variant analysis of obesity associated genes in young adults from a consanguineous population of Pakistan

Sadia Saeed[1,2], *Qasim M. Janjua[3], Attiya Haseeb[4], Roohia Khanam[4], Emmanuelle Durand[2], Emmanuel Vaillant[2], Lijiao Ninj[2], Alaa Badreddine[2], Mickaël Canouil[2], Mehdi Derhourhi[2], Amélie Bonnefond[2,1], Muhammad Arslan[4], Philippe Froguel[1,2]*

[1]Department of Metabolism, Digestion and Reproduction, Faculty of Medicine, Imperial College London, London, United Kingdom, [2]University of Lille, Inserm UMR 1283, CNRS UMR 8199, EGID, Institut Pasteur de Lille, Lille University Hospital, Lille, France, [3]University College of Medicine, The University of Lahore, Lahore, Pakistan, [4]School of Life Sciences, Forman Christian College, Lahore, Pakistan.

Introduction: Pakistan has one of the highest rates of consanguinity worldwide. Our previous studies in this population demonstrate an exceptionally high prevalence (30-49%) of monogenic obesity in children, mainly due to homozygous mutations in *LEP* and *LEPR* genes. Here, for the first time, we assess rare variants (MAF < 0.001 in gnomAD) in obesity associated genes in adults with severe obesity from the same region.

Methods: Genomic DNA from randomly selected 128 subjects (BMI = 37.7 ± 0.5; Age = 18.7 ± 0.3) was screened by conventional or augmented whole exome analysis.

Results: We identified thirteen subjects carrying 13 variants in 7 monogenic obesity genes (*LEPR*, P*CSK1*, *MC4R*, *NTRK2*, *POMC*, *SH2B1* and *SIM1)* of which 4 predicted (likely) pathogenic through ACMG criteria. We further identified a novel homozygous stop-gain mutation in *ASNSD1* gene, inactivation of which in mouse results in obesity. Additionally, we identified 10 homozygous mutations in genes previously linked to obesity-associated traits through GWAS. Finally, analyses of CNVs resulted in identification of 4 copy-loss variants.

Conclusions: Of significance is the identification of novel/rare variations in genes linked to obesity by GWAS and mouse knock-outs providing mechanistic leads to genetic identification of severe obesity in human. Whereas in this adult cohort variants in genes involved in the downstream leptin signalling appear to be more prevalent, inactivating mutations in genes encoding key regulators of leptin melanocortin pathway are absent (*LEP*) or under-represented (*LEPR*). This is presumably due to high pathogenicity and mortality risk, and social disadvantage, in children with LEP or LEPR deficiency. *Supported by MRC, ANR-10-LABX-46 and ANR-10-EQPX-07-01 (PF)*

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P13.033.B Performance benchmarking for calling and phasing of single-nucleotide polymorphisms and structural variants using Nanopore sequencing

*Rocío Esteban, Anthony Doran, Irina-Alexandra Vasilescu, Daniel J. Turner, David Stoddart, Sissel Juul, Eoghan Harrington, **Philipp Rescheneder***

Oxford Nanopore Technologies Ltd, Oxford, United Kingdom.

Single nucleotide polymorphisms (SNPs) and structural variants (SVs) are critical for our understanding of how genomic changes drive phenotypes. In the past there has been a strong focus on SNPs without taking into account haplotype or long-range information. With the advent of long read sequencing, this focus has been shifting towards larger SVs uncovering their significance across a broad range of fields, from diseases such as cancer to encoding desirable crop traits. Equally, the importance of haplotype information has become more apparent i.e., to identify compound heterozygous variant combinations as disease-causing candidates.Read lengths and coverage routinely obtained from Nanopore sequencing allow unique mapping across repetitive regions which are enriched in SVs. Furthermore, single reads can span large and complex variation end-to-end and cover multiple single nucleotide variants for phasing.To assess the performance of SV calling and read phasing with Nanopore sequencing, we sequenced the well characterised GM24385 cell line and compared the resulting SV and SNP calls against the Genome-In-A-Bottle truth set. We found high precision and recall for SNP calling (>99.5%) and SV calling (>95% and >97% respectively) and median phase block lengths of up to 2.6 Mbp. Furthermore, we elucidated the impact of read length, read depth, SV type and length on SV calling performance especially in repetitive regions and investigated what percentage of the human genome can be confidently phased.Finally, we illustrated how both SV calling and phasing can be combined and applied to complex regions of the human genome like the MHC locus.

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P13.034.C Characterization of disease associated tandem repeat regions in Slovenian population from exome sequencing data using Expansion Hunter

***Gaber Bergant**, Aleš Maver, Borut Peterlin*

Clinical Institute of Genomic Medicine, Ljubljana, Slovenia.

Variation in tandem repeat (TR) sequences is common in the human genome and normally not associated with human genetic disorders. However, large repeat expansions are increasingly recognized as an important cause of human genetic disorders. While point variants are well characterized across the human genome, population data on structural variants in TR regions remain lacking. For this reason, we analyzed exome sequencing data of 4.329 patients consecutively referred to our center for diagnostics of diverse rare genetic disorders. We used Expansion Hunter to estimate TR variation in 36 genes, where repeat expansions are associated with human genetic disorders. TR variation characterization in the Slovenian population yielded informative results for 73,893 alleles in 17 TR regions and was limited to coding, UTR, and several well-covered non-coding regions. Premutation was detected in 653 (0.88%) and full mutation in 27 (0.04%) alleles. The resulting TR profile is comparable to other previously published European cohorts. We provide the TR profile for comparison with other populations as well as for the use in diagnostics of rare genetic disorders as a source of background structural genetic variability. The results confirm that TRs represent an important source of human genetic variability which can be partly detected using ES, however, larger whole-genome sequencing studies are required for their genome-wide characterization.

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P13.035.D *De novo* mutation in ancestral generations evolves haplotypes contributing to disease

***Zeynep Hande Coban Akdemir**[1], Xiaofei Song[2], Davut Pehlivan[2], Ender Karaca[2], Yavuz Bayram[2], Francisco Ceballos[3], Tomasz Gambin[4], Shalini Jhangiani[2], Donna Muzny[2], Richard Lewis[2], Pengfei Liu[2], Eric Boerwinkle[1], Ada Hamosh[5], Richard Gibbs[2], V. Reid Sutton[2], Nara Sobreira[5], Claudia Carvalho[2], Jennifer Posey[2], Chad Shaw[2], Dave Valle[5], James Lupski[2]*

[1]UTHealth School of Public Health, Houston, TX, USA, [2]Baylor College of Medicine, Houston, TX, USA, [3]Instituto de Salud Carlos III, National Center of Microbiology, Madrid, Spain, [4]Warsaw University of Technology, Warsaw, Poland, [5]Johns Hopkins University School of Medicine, Baltimore, MD, USA.

We investigated the influences of admixture and consanguinity on the genetic architecture of disease by generating a variome derived from exome sequencing (ES) of 1,416 unrelated Turkish (TK) individuals, (643 unaffecteds, and 773 affecteds with various disease phenotypes. Clustering closely with European genomes, the TK population consists of two main subpopulations: compared to the first cluster (N = 285), the second cluster (N = 1,131) demonstrated a higher fraction of European and South Asian ($P = 1.57e^{-4}$ and $8.77e^{-7}$) and a lower fraction of Middle Eastern ($P = 8.78e^{-7}$) ancestry. Intriguingly, only 10% and 5% respectively of the first (N = 660,255) and second cluster (N = 1,845,686) variants are present in the Greater Middle Eastern (GME) variome, emphasizing the necessity of an independent TK control database (https://turkishvariomedb.shinyapps.io/tvdb/) for variant interpretation. Higher coefficient of inbreeding (F) values observed in the TK affecteds vs. unaffecteds (0.053 vs. 0.028, $P = 2.35e^{-18}$) manifested in an increased genome-wide burden of long-sized (>3.227 Mb) regions of homozygosity (ROHs) (114.24 vs. 59.14 Mb, $P = 8.77e^{-18}$), inferring 'young haplotypes', derived as *de novo* variant alleles in antecedent generations of the clan. These ROHs are enriched for ultra-rare, multi-locus, homozygous, deleterious variants and we hypothesize their combinatorial effects produce blended phenotypes accounting for the observed disease. Further, a retrospective analysis of previously published cases with >=2 disease loci (N = 69) revealed that those cases display significantly increased F values (0.062 vs. 0.038, $P = 2e^{-4}$) and total span of long-sized ROHs (186 vs. 141.78 Mb, P = 0.012). These data support a Clan Genomics model for disease in a population.

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P13.036.A Dissecting expansion dynamics and nucleotide variation in human-specific variable number tandem repeats

*Meredith M. Course, Arvis Sulovari, Kathryn Gudsnuk, Evan E. Eichler, **Paul N. Valdmanis***

University of Washington, Seattle, WA, USA.

There are over 55,000 variable number tandem repeats (VNTRs) in the human genome. However, their internal sequence composition and length variability amongst humans is largely unknown. Using long-read whole genome sequence information available from 32 phased individuals from the 1000 Genomes Project and Human Genome Structural Variant Consortium, we examined the internal sequence and length of >50 VNTRs, prioritized by those that are unique to humans or expand specifically in the human lineage. We find several examples of VNTRs that fall into one of three categories: 1) repeats that have identical internal repeat units, but which differ in length amongst individuals and geographical super-populations; 2) repeats with nucleotide substitutions at each copy of the repeat but with a defined order to these substitutions and 3) repeats with substantial diversity in both length and internal sequence of the repeat. Interrogating these repeats has revealed common patterns of repeat expansions and contractions across VNTRs. In addition, we find several tandem repeats that are significantly different in length between different 1000 Genomes Project super-populations. These findings build on our multiplexed long-read sequence analysis of a 69bp intronic repeat in *WDR7* in >300 individuals, which revealed multiple origins of the repeat that have continued to expand in a directional manner. Collectively, we are building a framework for understanding how repeats expand, how this information can be leveraged to help inform patterns of human migration, and mechanisms by which repeat expansions can lead to disease state.

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P14 Cytogenetics

P14.002.C 16p13.11p11.2 triplication syndrome: a new recognizable genomic disorder characterized by Bionano optical mapping and whole genome sequencing

Romain NICOLLE[1,2], *Karine Siquier-Pernet*[2], *Marlène Rio*[2,3], *Anne Guimier*[3], *Emmanuelle Olivier*[4], *Patrick Nitschke*[4], *Christine Bole-Feysot*[5], *Serge Romana*[1,2], *Alex Hastie*[6], *Vincent Cantagrel*[2], *Valérie Malan*[1,2]

[1]*Service d'Histologie Embryologie Cytogénétique, Hôpital Necker-Enfants Malades, Paris, France,* [2]*Developmental Brain Disorders Laboratory, Imagine Institute, Paris, France,* [3]*Département de Génétique Médicale, Hôpital Necker-Enfants Malades, Paris, France,* [4]*Bioinformatics Core Facility, Imagine Institute, Paris, France,* [5]*Genomics Platform, Imagine Institute, Paris, France,* [6]*Bionano Genomics, San Diego, CA, USA.*

Highly identical segmental duplications (SDs) account for over 5% of the human genome and are enriched in the short arm of the chromosome 16. These SDs are susceptibility factors for recurrent chromosomal rearrangements mediated by non-allelic homologous recombination (NAHR). Chromosomal microarray analysis (CMA) has been widely used as the first-tier test for individuals with neurodevelopmental disorders and several genomic imbalances involving the 16p-arm have been identified with this technic. However, the resolution of CMA and the limitations of short-reads whole genome sequencing (WGS) technology don't allow the full characterization of the most complex chromosomal rearrangements preventing a good understanding of the underlying mechanism. Herein, we report on two unrelated patients with a *novo* 16p13.11p11.2 triplication detected by CMA sharing a similar phenotype including hypotonia, severe neurodevelopmental delay with profound speech impairment and hyperkinetic behavior, chronic otitis media and distinctive facial features. Furthermore, the CMA also detected a 16p11.2 duplication which is approximately 450 kb in size for patient 1 and 300 kb in size for patient 2. Complete genetic characterization of these events was unreliable by WGS because the breakpoints lie within SDs. Consequently, we used Bionano optical mapping to fully characterize these chromosomal abnormalities. Thus, we propose a two-step mechanism to explain these rearrangements: a U-type exchange at a distal SD between homologous chromatids, and a NAHR event at BP1-BP3 region for patient 1 and BP1-BP2 region for patient 2. In conclusion, Bionano Genomics is a useful technology for unravelling the origin of complex chromosomal rearrangements involving SDs.

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P14.003.D The new chromosome 2p15-p13.2 microduplication syndrome: a case report

Dario Di Salvio[1], *Maria Tessitore*[2], *Maria Anna Siano*[2], *Mariateresa Falco*[3], *Piero Pignataro*[4], *Rita Genesio*[4], *Daniela Melis*[2]

[1]*A.O.U. Federico II, Naples, Italy,* [2]*Department of Medicine, Surgery and Dentistry, Scuola Medica Salernitana, University of Salerno, Salerno, Italy,* [3]*Pediatric Unit San Giovanni di Dio e Ruggi d'Aragona University Hospital, Salerno, Salerno, Italy,* [4]*Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples, Italy.*

Introduction: Microdeletions of various sizes in the 2p16.1-p15 chromosomal region have been assembled together under the 2p16.1-p15 microdeletion syndrome. Children with this syndrome usually share features including microcephaly, developmental delay, feeding problems, facial dysmorphism (e.g. epicanthus, ptosis, bitemporal narrowing, telecanthus), urogenital and skeletal abnormalities. We present a child with an interstitial 11,3 Mb duplication of 2p15-p13.2.Case report. We report a 11-months-old girl with neurodevelopmental delay, hypotonia and minor dysmorphic features (epicanthal folds, left eye ptosis, broad nasal root, rounded forehead, retrognathy and preauricolar appendages). A chromosomal microarray analysis demonstrated a microduplication of 11,3 Mb on chromosome 2p15-p13.2, containing 99 OMIM genes.

Conclusions: Clinical features of patients with microduplication of this region have been described in few reports in literature and rarely registered in laboratory databases. These are distinct from those of children with the 2p16.1-p15 microdeletion syndrome: specifically in few cases is described the presence of hypotonia, the head circumference is within the normal range and the neurodevelopmental delay is less severe. BCL11A, USP34 and PEX13 genes of the 2p16.1-p15 region have been usually described as involved in neurodevelopmental delay in children with microduplication of 2p16.1-p15 region; these genes are not involved in this region, although our patient shares phenotypic characteristics with those affected by 2p16.1-p15microduplication. The genes UGP2, MDH1, ASCT1, EMX1, and EXOC6B, comprised in the region described in this report, are involved in neurodevelopment and they could be responsible for the neurological phenotype.

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P14.004.A Duplication of 8p23.1 detected in a prenatal cytogenetic study

Rosa Martorell[1], *Albert Tubau*[2], *Jordi Rosell*[1], *Victor Asensio*[1], *Carme Vidal*[1], *Laura Torres*[1], *Damià Heine*[1], *Antonia Obrador*[1], *Iciar Martinez*[1]

[1]*Hospital Universitari Son Espases, Palma de Mallorca, Spain,* [2]*Hospital Universitari Son LIàtzer, Palma de Mallorca, Spain.*

Duplications of 8p23.1 have been associated with a wide variety of presentations from euchromatic variants to developmental delay and heart disease. Here, we present a de novo duplication of 8p23.1 found at a prenatal cytogenetic diagnosis. A 32-year-old woman was referred for prenatal diagnosis for cervical incompetence. Before a cervical cerclage we performed a routine cytogenetics study. We found an apparent duplication of 8p23.1 in the amniotic fluid cells on G-banding. The appearance of cytogenetic duplication was seen as a fine G-dark band at the center of an expanded G-light 8p23.1 band. Both parents had normal karyotype. We investigated the region using oligonucleotide array Comparative Genomic Hybridization (aCGH). The study showed a ≈5Mb genomic duplication of band 8p23.1 that did not run into euchromatic variants and it modified the dosage of SOX7 and GATA4 genes.Patients with duplication of band 8p23.1 show a diversity of clinical findings.There are debates about the relationship of the duplicated GATA4 gen and congenital genetic defects in these patients. Long et al., 2013 published the first evidence that the duplication of SOX7 gen has a strong association with heart defects, while in 2015 Barber et al., found that the variable expressivity in patients with duplications 8p23.1 should be caused by the duplication and interactions of the SOX7 and GATA4 transcription factors. However, in our case, congenital cardiac defects were not observed by fetal ultrasound. After genetic counselling the parents chose to continue their pregnancy and at this moment the pregnancy is continuing.

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P14.005.B Cytogenetic profile of newly diagnosed patients with acute myeloid leukemia - a single centre retrospective study

Dinnar Yahya[1], *Tsanka Ruseva*[2], *Mari Hachmeriyan*[1], *Lyudmila Angelova*[3], *Ilina Micheva*[1], *Trifon Chervenkov*[1]

[1]*UMHAT "St. Marina", Varna; Medical University "Prof. Dr. Paraskev Stoyanov", Varna, Bulgaria,* [2]*UMHAT "St. Marina", Varna, Bulgaria,* [3]*Medical University "Prof. Dr. Paraskev Stoyanov", Varna, Bulgaria.*

Introduction: Acute myeloid leukemia(AML) is a heterogeneous group of disorders, seen predominantly in adults. It originates from abnormally differentiated myeloid progenitors as a result of numerous genetic events. AML is characterized by wide genetic heterogeneity, complex pathogenesis and variable survival rate. Identification of cytogenetic abnormalities at diagnosis is important for classification, prognostification, treatment choice and response determination.

Materials and methods: We conducted a retrospective study on newly diagnosed adult patients with AML who underwent conventional cytogenetic analysis(CCA) in the Laboratory of Medical genetics, Varna between 01.2019-12.2020. A total of 74 patients were tested using bone marrow samples and G-banding technique. CCA was performed accordingly with the *International System for Human Cytogenomic Nomenclature 2016*.

Results: Karyotyping was successful in 63 (85.1%) of the evaluated patients with 11 (14.9%) samples lacking metaphase plates. CCA showed normal karyotype(NK) in 34 (54%), and abnormal karyotype in 29 (46%) cases. According to the *European LeukemiaNet risk stratification 2017*, 6 (9.5%) of the patients were with favorable, 44 (69.8%) with intermediate, and 13 (20.6%) with adverse risk. The two-year study showed overall survival of 64%, 24% and 10%, respectively, which correlated with the risk groups.

Conclusions: CCA is a basic method in AML diagnosis, incorporated in classification and risk stratification. Due to technical problems the method is not always informative. Also, given the known molecular genetic markers, significant for diagnosis, prognosis and monitoring, it is highly recommended to combine karyotyping with molecular genetic analysis. This is particularly important in cases of unsuccessful CCA or NK where additional clarification is essential.

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P14.006.C Constitutional chromosomal abnormalities over 28 years services at King Abdulaziz Medical City tertiary medical center, Riyadh, Saudi Arabia

Mohammed A. AlBalwi[1,2,3], *Aziza Alkhaldi*[1], *Dina Hommam*[1], *Mohammmed Adam*[1], *Alaa AlMeleih*[1], *Abdulelah Abunadi*[1], *Salman Alsaad*[1], *Yazeed Althobaiti*[1], *Hala Alomair*[1], *Ghady Alfaggy*[1], *Barak Alawad*[1], *Alwaleed Almalki*[1], *Ashwag Alghamdi*[1], *Bader Almuzzani*[1,3], *Nasser Alatwi*[1]

[1]*King Abdulaziz Medical City, Riyadh, Saudi Arabia,* [2]*King Saud bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia,* [3]*King Abdullah International, Medical Research Center, King Saud bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia.*

Introduction: Despite advances molecular diagnostic techniques such as aCGH, WES and WGS, cytogenetic chromosomal analysis still the golden standard test for detection most of the genetic abnormalities.

Materials and Methods: A retrospective review of 15,836 referred cases over a period of 28 years (1992-2020) were diagnosed with standard karyotyping analysis for constitutional chromosomal abnormalities at Cytogenetics laboratory, Department of Pathology and Laboratory medicine at King Abdulaziz Medical City, Ministry of National Guard - Health Affairs, Riyadh.

Results: Constitutional chromosomal aberrations were detected in 2081 cases, with 13.14% overall positive rates of abnormal cytogenetic findings (2081/15,836). Among the cases with chromosomal aberrations, 1616 (77.7%) were numerical abnormalities and 465(22.3%) were structural abnormalities. For the numerical abnormalities: 4 cases (0.24%) were with triploidy; 1144 (70.8%) with trisomy 21; 133 (8.23%) with trisomy 18; 86 (5.32%) with trisomy 13; 9 (0.56%) with mosaic autosomal trisomy; 66 (4.1%) with 45,X; 91 (5.6%) with 47,XXY; 12 (0.74%) with 47,XXX; 6 (0.37%) with 47,XYY; and 65 (4.0%) with a mosaic sex chromosome aberration. For the structural abnormalities: 132 cases (28.3%) were with reciprocal translocation; 28 (6.0%) with Robertsonian translocation; 33 (7.1%) with inversion; 158 (34%) with deletion; 31 (6.7%) with duplication; 50 (10.8%) with isochromosome; 14 (3.0%) with ring chromosome; and 19 (4.1%) with marker chromosome.

Conclusions: Our result has presented the largest series of constitutional chromosomal cases in Saudi Arabia that might help in better patient care management and better counseling for the affected families for future family planning.

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P14.007.D Clinical findings on chromosome 1 copy number variations

Filipa Leitão[1], *Joel Pinto*[1,2], *Carolina Almeida*[1,2], *Vera Lima*[1,2], *Armanda Passas*[3], *Miguel Leão*[4], *Ana Grangeia*[4,1,2], *Sofia Dória*[1,2]

[1]*Department of Pathology, Genetics Service, Faculty of Medicine - University of Porto, Porto, Portugal,* [2]*I3S-Instituto de Investigação e Inovação em Saúde, University of Porto, Portugal, Porto, Portugal,* [3]*Neurodesenvolvimento- UNIA, Centro Hospitalar Vila Nova de Gaia/ Espinho – CHVNG, Vila Nova de Gaia, Portugal,* [4]*Clinical Genetics Service, Centro Hospitalar de são João, Porto, Portugal.*

Background: Copy number variants (CNVs) are a major contribution to genome variability. The presence of CNVs on chromosome 1, the largest human chromosome, is a known cause of morbidity. The main objective of this study was to contribute for chromosome 1 disease map, through the analysis of patients with chromosome 1 CNVs.

Methods: Using the array-CGH database of the Department of Genetics of the Faculty of Medicine, University of Porto, patients with a pathogenic (P) or probably pathogenic (VOUS-PP) CNVs on chromosome 1 detected by array-CGH were included in the study. Clinical information was collected for all patients. Databases and related literature were used for a better understanding of the genotype-phenotype correlation.

Results: From a total of 2380 patients included in the database we identified 24 patients (1,0%) with chromosome 1 CNVs, P (9 cases) or VOUS-PP (15 cases). These CNVs account for 7.1% (24/341 CNVs) of the total P/VOUS-PP CNVs included in the database. The most common CNVs found were on 1q21.1 (either deletions or duplications), with some of them also spanning the 1q21.2 region. Four patients presented additional CNVs on chromosomes 8, 16 and 17.

Conclusion: This study reinforces the association between chromosome 1 CNVs and neurodevelopmental disorders/craniofacial dysmorphisms. However, it also strengthened the idea that not always the interpretation of CNVs and the genotype-phenotype correlation is a linear task since a wide spectrum of variants can be identified between benign CNVs and clearly pathogenic CNVs.

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P14.010.C From cytogenetics to cytogenomics - what is the underlying idea?

Thomas Liehr

Jena University Hospital, Institute of Human Genetics, Jena, Germany.

Research and diagnostics in human, with chromosomes in focus, were originally designated as "cytogenetics". Working with human chromosomes rather than DNA was very popular in the 1970s/1980s. However, latest since ~1990s, mainstream human molecular geneticists looked at people dealing with chromosomes as something like 'outdated fossils'. Interestingly, this attitude was never justified by any evidence, and it is imperative to understand, that all available techniques to study the human genome - at different levels of resolutions, and at level of the single cell or by approaching millions of cells at time - rather complement, than play against each other. Cytogeneticists feel driven by a not-real(?) pressure from field, and (over)reacted in parts by changing well-established designations from "cytogenetics" to "cytogenomics", as: the "international system of cytogenetic nomenclature (ISCN)" was renamed to "international system of cytogenomic nomenclature" in 2016, "American Cytogenetics Conference" to "American Cytogenomics Conference" in 2018, "European Cytogenetics Conference" to "European Cytogenomics Conference" in 2019. Astonishingly, no definition is given for cytogenomics, neither in ISCN nor anywhere in literature. Here we provide a definition for cytogenomics, which has a comprehensive and integrative view. Accordingly, cytogenomics is equivalent to what was defined as "chromosomics" by Uwe Claussen (Jena-Germany) in Cytogenet Genome Res. 2005). His idea was, to subsume under such a term all chromosome-related research with the goal to lead us to novel concepts in biology. Cytogenomics should be used in future as a genome- and chromosome-oriented term, with the goal to describe research, rather than technical approaches.

T. Liehr: None.

P14.011.D Down syndrome with an inherited translocation t (6;21)(q13;q22)

Rawia Kammoun[1], *Imene Boujelbene*[1], *Ikhlas Ben Ayed*[1], *Nourhene Gharbi*[1], *Mohamed Ali Ksentini*[1], *Ines Ouertani*[2], *Fatma Abdelhedi*[1], *Hassen Kamoun*[1]

[1]*Medical Genetics Department, Hedi Chaker Hospital, sfax, Tunisia,* [2]*Department of Congenital and Inherited Disorders,Charles Nicolle Hospital, Tunis, Tunisia.*

Background: In less than 5% of cases, Down syndrome (DS, OMIM#190685) is due to translocation.The most common translocations observed are between the long arms of chromosome 21 and another acrocentric. Few translocations involving other chromosomes have been reported. We report a rare case of DS due to a balanced maternal t(6;21)(q13;q22).

Clinical report: A 3-year-old girl was referred with her parents for genetic counseling. She was the first child of healthy non-consanguineous parents. Her mother, with a history of three unexplored spontaneous abortions, had 30 years old at conception. The proband was born after a poorly followed pregnancy. DS was clinically suspected at birth. The growth is normal and motor development was delayed. Biological and radiological investigations were normal. Karyotype confirmed the diagnosis and showed 47,XX,t(6;21)(q13;q22),+21 which was inherited from a balanced maternal translocation.Prenatal diagnosis was indicated in the next pregnancy and the fetal karyotype was normal (46,XX).

Discussion: This is the first report of DS due to an inherited t (6;21). Balanced parental translocations involving chromosome 21 are responsible for most familial cases of DS. In this case, 3-to-1 disjunctional segregation has occurred. Other possibilities of segregation are possible and can give non-viable fetus which explained the spontaneous abortions.The recurrence risk for DS due to an inherited translocation is difficult to evaluate and it is about 10%. Thus, prenatal diagnosis should be offered to these families. We highlight the importance of determination of cytogenetic mechanisms in DS that provides the appropriate genetic counseling.

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P14.013.B Chromatid cohesion defect in a patient with pathogenic variant of the FAM111A gene

Marta Rusmini, *Simona Cavani, G.A. Rotulo, F Caroli, D Coviello, Isabella Ceccherini, Maja Di Rocco*

Ist G. Gaslini, genova, Italy.

Pathogenic heterozygous mutations of the *FAM111A* gene are reported in both osteocraniostenosis (GCLEB; OMIM #602361) and Kenny Caffey syndrome type 2 (KCS2; OMIM #127000), however the function of this gene and its role in the cellular biology is still unknown. The cytogenetic examination of an infant with pre and postnatal growth failure, normal psychomotor development, skeletal abnormalities and idiopathic hypercalciuria causing nephrocalcinosis, nephrolithiasis and brain calcifications, has incidentally identified an unexpected defect of chromatids cohesion during metaphase. Because the patient's clinical phenotype was not consistent with known cohesinopathies, a whole exome sequencing was performed, and a *de novo* FAM111A missense variant identified, showing a frequency <10E-5, affecting a highly conserved codon, neighbor to codons bearing either KCS2 or GCLEB causative changes, and predicted as pathogenic. Our findings expand the clinical phenotype associated with FAM111A mutations beyond KCS2 and GCLEB and, consistent with FAM111A interaction with the Proliferating cell nuclear antigen (PCNA) and its re-localization to chromatin during the S-phase, suggest a role of this gene as chromatin replication factor and of the present associated phenotype as a novel coesinopathy.

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P14.014.C Frequency of aneuploidy in diploid androgenetic hydatidiform moles

Pernille Walbum Kristensen[1,2], *Lotte Andreassen*[3], *Marianne Geilswijk*[3], *Thomas Poulsen*[2], *Isa Niemann*[3], *Lone Sunde*[1,2]

[1]*Aalborg University Hospital, Aalborg, Denmark,* [2]*Aarhus University, Aarhus, Denmark,* [3]*Aarhus University Hospital, Aarhus, Denmark.*

Introduction: A hydatidiform mole is a non-viable conceptus, many are diploid and with androgenetic genome. In some cases, the genome is homozygous, as if it originates from one spermatozoon. In other cases, the genome is heterozygous, often from two spermatozoa. One recent study has observed a higher frequency of aneuploidy in heterozygous, compared to homozygous diploid androgenetic hydatidiform moles (Scientific Reports (2020) 10:17137).

Methods: During a 35-year period, samples were collected from >800 conceptuses, suspected to be hydatidiform moles by gynecologists. Ploidy was estimated by karyotyping. Parental origin of the genome was determined by analyzing 9-31 polymorphic loci. Inclusion criteria were diploidy, successful karyotyping, and androgenetic origin of the genome. Multiple pregnancies and mosaics were excluded.

Results: Among 209 (near) diploid androgenetic hydatidiform moles, 176 were homozygous, and 33 heterozygous. Among homozygous hydatidiform moles 0 showed aneuploidy. Among heterozygous hydatidiform moles 3 showed aneuploidy (9%). A significantly higher frequency of aneuploidy was shown in diploid androgenetic heterozygous hydatidiform moles, compared to diploid androgenetic homozygous hydatidiform moles ($p = 0.005$).

Conclusion: Our results support, that in diploid androgenetic hydatidiform moles, the frequency of aneuploidy is higher in conceptuses showing heterozygosity, than in those showing homozygosity. A part of the explanation may be related to the number of centrioles. In the zygote, the centrioles originate from the spermatozoon. Thus, in a diploid androgenetic homozygous hydatidiform mole there should be one pair of centrioles, in a heterozygous hydatidiform mole there should be two. Two pairs of centrioles might disturb the segregation of chromosomes, causing aneuploidy.

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P14.015.D Incidental finding of DFNB1 locus deletion carriers associated with non-syndromic deafness after prenatal analysis in amniotic fluid

Matías Pérez, *María Luz Bellido, Teresa de Haro*

Hospital Universitario Virgen de las Nieves, Granada, Spain.

Introduction: Deletions in DFNB1 locus at chromosome 13q11-q12 are uncommon in most populations. This locus includes *GJB2* and *GJB6* genes that express connexins at the cochlea. We present the case of parents carriers with heterozygous deletions in the DFNB1 locus encompassing different parts of the *GJB6* and *CRYL1* genes and the putative regulatory region of the *GJB2* gene, discovered after array-CGH analysis in amniotic fluid (AF) from its fetus.

Methods: Array-CGH analysis was performed by a CGX™ HD v1,1 4-plex array 180 k (PerkinElmer), with an average resolution of 40 kb in the backbone and 20 kb in the regions of interest.

Results:

DFNB1 locus could present two different size deletions. The most common deletion with a size of 309 kb encompasses *GJB6* (from exon 1) and *CRYL1* gene, and the second one of 232 kb encompasses from *GJB6* intron 5 to intron 4 of *CRYL1*, both present in the fetus.

Conclusion: Incidental finding are growing in importance since the appearance of new technologies. These incidental findings are clinically relevant in counseling genetic for the family for future pregnancies and it is also necessary to consider the medical benefit for the clinical care of patient.

M. Pérez: None. **M. Bellido:** None. **T. de Haro:** None.

P14.016.A Cytogenetic and Y chromosome microdeletion analysis in infertile male patients with azoospermia. A hospital based lab experience in Karachi, Pakistan

MUHAMMAD ISRAR NASIR, *Ijaz Ahmad, Muhammad Ammad*

LIAQUAT NATIONAL HOSPITAL AND MEDICAL COLLEGE, KARACHI, Pakistan.

Background: Infertility was found to affect approximately 10%-15% of the couples, worldwide. The chromosomal abnormality is more common in infertile men. The aim of this study was to evaluate the frequency and type of chromosomal abnormalities and Y-chromosome Micro-deletions in the infertile men with Normal Chromosomal Component.

Materials and Methods: A total of 105 infertile males, with azoospermia, were included in this prospective observational study. Samples were collected from the infertile males, and examined by Karyotype analysis. Males who were found having Normal 46,XY Karyotype were further tested for Y chromosome micro-deletion by PCR. Phenotypic Features like Height, Weight, Head Circumference were recorded. Other information gathered were ethinic group and marriage information. Informed Consent was collected from each individual.

Results: Among the 105 infertile patients, (19%) exhibited chromosomal abnormality, including 13 (12.3%) subjects with

typical Klinefelter syndrome, Four (3.8%) with the autosomal chromosome abnormality. Two cases showed (4.76%) two cells lines Normal Chromosomal component with 47, XXY. One Patient demonstrated 46,XX PHENOTYPIC MALE Karyotype. Of 85 Individuals with Normal Karyotype 46,XY, Further testing for Y-Chromosome Micro-deletion reveled 4 individuals with AZFC and One individual with AZF b+c deletion.

Conclusion: The results from this study demonstrated that chromosomal abnormalities are common in the infertile men with a higher frequency of sex chromosomal abnormality, especially those with the numerical type. This study also highlights the importance of Cytogenetic findings in decision making and better management of the patients in infertility clinics.

M.I. Nasir: None. **I. Ahmad:** None. **M. Ammad:** None.

P14.017.B Familial segregation analysis of copy number variants: a crucial role of FISH (over array CGH)

Irene Mademont-Soler[1], *Carles Garrido*[2], *Dolors Casellas-Vidal*[1], *Javier Nieto-Moragas*[1], *Susanna Esteba-Castillo*[3], *Ariadna Cherino*[1], *Núria Cortés*[1], *Elvira Vilà*[1], *Ana Morales*[2], *María José Naharro*[2], *Miquel Àngel Caamaño*[2], *Xavier Queralt*[1], *María Obón*[1]

[1]*Hospital Universitari de Girona Dr. Josep Trueta, Girona, Spain,* [2]*Reference Laboratory, Hospitalet de Llobregat, Spain,* [3]*Institut d'Assistència Sanitària, Salt, Spain.*

Introduction: Constitutional insertional translocations are a rare finding in clinical cytogenetics, but their unbalanced forms may lead to intellectual disability and/or other congenital anomalies. In the last years, it has been suggested that these chromosomal rearrangements are more frequent (1:500) than previously thought (1:80000).

Materials and Methods: A family with an insertional translocation (inherited in both balanced and unbalanced forms) is presented. The techniques used for its characterization included array CGH, FISH and high resolution karyotyping.

Results: Array CGH performed in two sisters with moderate intellectual disability, macrocephaly and skeletal anomalies identified, in both of them, a deletion of 8.6 Mb of chromosome 10q23.1q23.31. Family FISH studies (together with high resolution karyotyping) revealed that the mother and a healthy brother presented an insertion of 10q23.1q23.31 into 6q1?3. Thus, the deletion identified in both sisters was the result of an unbalanced segregation of a balanced maternal rearrangement.

Conclusions: Segregation studies of copy number variants are often performed exclusively by array CGH. Such approach may lead to misdiagnosis in some families, as balanced rearrangements (such as insertional translocations), cannot be detected by this technique. Our results strongly support once more the need for FISH and conventional karyotyping to resolve adequately these cases. The recognition of familial balanced chromosomal rearrangements is crucial for genetic counselling, because if they are inherited the recurrence risk changes radically. Of note, such characterization could also be performed by whole-genome sequencing, although this technique is not routinely used nowadays for the study of these families.

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P14.018.C Three additional marker chromosomes in a girl with global developmental delay and microcephaly

Ilona Dietze-Armana[1], *Maren Wenzel*[1], *Thomas Liehr*[2], *Karl Mehnert*[1]

[1]*genetikum, Neu-Ulm, Germany,* [2]*Jena University Hospital, Friedrich Schiller University, Institute of Human Genetics, Jena, Germany.*

Introduction: Small supernumerary marker chromosomes (sSMC) are defined as additional centric chromosome fragments too small to be characterized unambiguously by banding cytogenetics alone (ISCN 2020). Further techniques are required for identification like molecular karyotyping or different methods of molecular cytogenetic. Live birth rate of sSMC is 0.05%, but incidence in children investigated for developmental delay is 0.3% (http://cs-tl.de/DB/CA/sSMC/0-Start.html). Case report: We report about an 18 month old girl with global developmental delay, muscular hypotonia, microcephaly and mild facial dysmorphisms. Family history was unremarkable.

Results: Because of muscular hypotonia SMA was excluded. Chromosome analysis of peripheral blood revealed an unbalanced karyotype with two abnormal cell lines. First, two nonmosaic alike bisatellited sSMC were found. Second, an additional ringchromosome in mosaicism was detected. Molecular karyotyping showed no genomic imbalance. We characterized the first two markers by FISH as inv dup(15). The other sSMC was characterized by subcenMFISH. This investigation showed the origin from chromosome 1. Karyotype: mos 49,XX,+inv dup(15)(q11.2)x2,+r(1)(p1?1.2q22.?1)[5]/48,XX,+inv dup(15)(q11.2)x2[35]. Furthermore, no UPD(15) was detected and gene panel analysis was normal. Parental chromosome analysis showed paternal origin with one inv dup(15), karyotype: mos 47,XY,+inv dup(15)(q11.1)[23]/46,XY[7], whereas the ringchromosome originated de novo.

Conclusion: Despite low level mosaicism we suggested that ringchromosome r(1) is cause of the phenotype of our patient. However, clinical findings are heterogeneous in relation of size, material in p or q arm and mosaicism/nonmosaic.

I. Dietze-Armana: None. **M. Wenzel:** None. **T. Liehr:** None. **K. Mehnert:** None.

P14.019.D A de novo pericentric inversion of chromosome 17 disrupting the *PAFAH1B1* gene in a patient with Miller-Dieker lissencephaly syndrome

Fatma Maazoun[1,2], *Imène Boujelbene*[1,2], *Nourhène Gharbi*[1,2], *Mohamed Ali Bouhlel Bouhlel*[1,2], *Sahar Aouichaoui*[1], *Sofiene Hentati*[1], *Fatma Kammoun*[3], *Chahnez Triki*[3], *Hassen Kamoun*[1,2], *Fatma Abdelhedi*[1,2]

[1]*Medical Genetics Department, Hedi Chaker Hospital, Sfax, Tunisia,* [2]*Human Molecular Genetics Laboratory, Faculty of Medicine of Sfax, Sfax, Tunisia,* [3]*Child Neurology Department, Hedi Chaker Hospital, Sfax, Tunisia.*

Background: Miller-Dieker Lissencephaly Syndrome (MDLS, OMIM#247200), is characterized by classic lissencephaly, dysmorphic features and/or many other congenital anomalies. MDLS is caused by microdeletions containing at least two genes, *PAFAH1B1* and *YWHAE*, mapped on the 17p13.3 region, while isolated lissencephaly can result from heterozygous mutation or deletion of *PAFAH1B1*.

Clinical report: We report the case of a 7-month-old boy, referred to genetic counselling because of west syndrome and lissencephaly. He was the third child of healthy non-consanguineous parents. Physical examination showed that growth and head circumference were on the mean curve. Some characteristic dysmorphic features were noted, including bitemporal narrowing, small eyes, epicanthus, depressed nasal bridge, small nose with anteverted nostrils and micrognathia. Neurological examination showed axial hypotonia, peripheral hypertonia and increased deep tendon reflexes. Cardiac and abdominal ultrasound showed no abnormalities, whereas brain MRI documented classic lissencephaly.

Results: Cytogenetic analyses were performed on cultured peripheral blood lymphocytes. The karyotype revealed a pericentric inversion of chromosome 17: 46,XY,inv(17)(p13.3;q23). Parental karyotypes were normal indicating that this rearrangement occurred de novo. FISH analysis showed that *PAFAH1B1* was disturbed by the breakpoints of this structural variant.

Discussion: It is well documented that *PAFAH1B1* haploinsufficiency is responsible for isolated lissencephaly, however the patient described here shares features with MDLS which might be explained either by lack of expression (position effect) or by deletion of other genes located in 17p13.3 region. In this regard, further genetic studies, such as RT-qPCR and array CGH will be performed leading to better genotype phenotype correlations.

F. Maazoun: None. **I. Boujelbene:** None. **N. Gharbi:** None. **M. Bouhlel:** None. **S. Aouichaoui:** None. **S. Hentati:** None. **F. Kammoun:** None. **C. Triki:** None. **H. Kamoun:** None. **F. Abdelhedi:** None.

P14.020.A Tissue-specific monosomy 13 in a patient with hemihypertrophy, facial dysmorphism, short digits and optic nerve colobomas

David Bohanna[1], *Claire Vince*[1], *Natalie Harper*[1], *Lucy Osland-Jones*[1], *Donna Evans*[1], *Susan Hamilton*[1], *Dominic McMullan*[1], *Nicola K. Ragge*[2,3]

[1]*West Midlands Regional Genetics Laboratory, Birmingham Women's and Children's Foundation Trust, Birmingham, United Kingdom,* [2]*West Midlands Regional Clinical Genetics Service and Birmingham Health Partners, Birmingham Women's and Children's Foundation Trust, UK, Birmingham, United Kingdom,* [3]*Faculty of Health and Life Science, Oxford Brookes University, Oxford, United Kingdom.*

Mosaic monosomy 13 is a rare cytogenetic finding associated with a variable phenotype that includes growth deficiency, microcephaly, brain malformations, facial dysmorphism, hand and feet defects, and genitourinary anomalies. Diagnosis is often delayed due to a normal result in the lymphocytes.

We describe a girl who presented with body asymmetry, plagiocephaly, microcephaly, myelomeningocoele, cradle cap, digital and skeletal anomalies, hypertelorism and optic nerve colobomas during the neonatal period. Subsequently she was diagnosed with a small cerebellum, left sensorineural deafness, right conductive deafness, wide-spaced nipples, smaller left kidney, sparse hair and delayed development. Combined microarray and karyotype of a blood sample was normal.

Clinical suspicion of mosaic monosomy 13 was raised following a case report in the literature, and prompted analysis of a skin biopsy. Microarray analysis detected a ~84Mb deletion of chromosome 13, likely to be present in a significant proportion of cells. Confirmatory chromosome analysis showed a 46,XX,del (13)(q12.3) karyotype. The deletion encompasses the majority of chromosome 13, consistent with a diagnosis of mosaic monosomy 13. In addition to providing a diagnosis, and a low recurrence risk for future pregnancies, this result is associated with a raised tumour risk, most notably for retinoblastoma, osteosarcoma and other tumours requiring life-long surveillance.

The clinical diagnosis of mosaicism may be under recognised, and should be considered in individuals displaying some of these features, particularly body asymmetry. This paper also highlights the possibility that other individuals with multiple developmental anomalies, including eye anomalies may have an underlying diagnosis of tissue-limited mosaicism.

D. Bohanna: None. **C. Vince:** None. **N. Harper:** None. **L. Osland-Jones:** None. **D. Evans:** None. **S. Hamilton:** None. **D. McMullan:** None. **N.K. Ragge:** None.

P14.021.B 47,XXY/46,XX/46,XY mosaic Klinefelter Syndrome accompanied by mixed connective tissue disorder: A very rare case

Aysel Kalayci Yigin, ***Mustafa Tarik Alay****, Deniz Agirbasli, Mehmet Seven*

Department of Medical Genetics, Cerrahpasa Medical Faculty, Istanbul University-Cerrahpasa, Istanbul, Turkey.

Klinefelter Syndrome (KS) is the most frequent chromosomal disorder in males. KS is mainly characterized by tall stature, eunuchoid habitus, narrow shoulders, small testes, gynecomastia, and infertility. Biochemical analysis usually shows low serum testosterone, high serum follicle-stimulating hormone (FSH), and luteinizing hormone (LH) levels with impaired spermatogenesis. Mixed connective tissue disorder (MCTD) is a systemic, autoimmune disease characterized by overlapping signs and symptoms of systemic lupus erythematosus, systemic sclerosis, polymyositis. The disease is least common in males compared to females. Here, we report the first time 47,XXY[83]/46,XX[4]/46,XY[13] mosaic Klinefelter syndrome with the MCTD. Only 3 cases were reported as 47, XXY regular type KS with MCTD, so far. Our case is a 50-year-old male referred to our department with lower extremity rash, persistent fever, arthralgia, muscle weakness, dry eye and mouth, and Raynaud's phenomenon. In physical examination, eunuchoid body, sparse axillary and pubic hair growth, small testes, bilateral gynecomastia, digital ulcers, telangiectasias, and splenomegaly were detected. Chromosome analysis of the patient revealed an abnormal karyotype of 47,XXY[83]/46,XX[4]/46,XY[13]. FISH analysis indicated that ish(SRYx1),(DZYx1)(DZX1x2)[92/100]/ish (SRYx0),(DYZ1x0)(DZX1x2)[5/100]/ish(SRYx1), (DZYx1)(DZX1x1) [3/100]. Autoimmune diseases are more common in females. It might be associated with escape from X-inactivation in early embryogenesis. Hence, due to the gene dosage effect, X-overexpression may cause an increased gene dosage and that may cause susceptibility to autoimmune disease. Due to the KS patients having an extra X-chromosome it might be

	array[hg19]	size	Avg value	Gene deleted	Status	Heritage	Classification	
Father	13q12.11(20,797,139-21,097,970)x1.	300,83 kb	-0,918	GJB6 and CRYL1	Ht	AR	Pathogenic	
Mother	13q12.11(20,804,067-21,032,170)x1.	228,10 kb	-0,964	Partial deletion of GJB6 and CRYL1	Ht	AR	Pathogenic	
AF fetus	13q12.11(20,798,175-20,803,032)x1	6,87 kb	-1,403	GJB6 and *CRYL1*	Hm	AR	Pathogenic	
								-6,477
								-1,310

explained with a similar mechanism. This rare finding's contribution to the literature is notable.

A. Kalayci Yigin: None. **M.T. Alay:** None. **D. Agirbasli:** None. **M. Seven:** None.

P14.022.D More accurate penetrance estimates for neurosusceptibility loci lead to significantly reduced penetrance estimates

Shuxiang Goh[1], *Rhys Bowden*[2], *Mark Pinese*[3], *Edwin Kirk*[1]

[1]*Sydney Children's Hospital, Sydney, Australia,* [2]*Monash University, Melbourne, Australia,* [3]*University of New South Wales, Sydney, Australia.*

Introduction: Penetrance has been defined as the proportion of individuals with a given genetic change who display a phenotypic change. We show that this is an ambiguous definition that leads to two different interpretations. One interpretation is used by clinicians and counsellors (essentially 'the likelihood that the variant will cause a phenotype'). A different mathematical interpretation has been used to calculate penetrance based on Bayes' theorem (essentially 'the likelihood the variant may been seen in association with a phenotype'). The latter definition encompasses individuals who have the variant by chance - associated with but not causal of the phenotype. The interpretations are incompatible. As a result, many of the published penetrance estimates for neurosusceptibility loci, intellectual disability, schizophrenia and autism are overestimated.

Methods: We provide a mathematical rationale for a more clinically-meaningful formula for estimating penetrance. We also improve the data used in the formula. We justify our approach in changing the background rate of any physical or intellectual disability from 5.12% used by some authors, to 1.1% for intellectual disability. This results in penetrance estimations for neurosusceptibility loci that are approximately 5-fold lower in some instances.

Results: When the two approaches are combined, we find that the penetrance for most neurosusceptibility loci are markedly decreased. Some previously low-penetrant neurosusceptibility loci are recalculated as having a penetrance of 0%.

Conclusion: Most neurosusceptibility loci have a lower penetrance than previously estimated. This has potential diagnostic and counselling implications in both the prenatal and postnatal settings.

S. Goh: None. **R. Bowden:** None. **M. Pinese:** None. **E. Kirk:** None.

P14.023.D Characterization of breakpoint regions of apparently balanced translocations by optical genome mapping

Jenny Schiller[1], *Karolina Bilska*[1], *Uwe Heinrich*[1], *Eva-Maria Krimmel*[1], *Stephanie Demuth*[2], *Imma Rost*[1]

[1]*MVZ Martinsried, Martinsried, Germany,* [2]*Mitteldeutscher Praxisverbund Humangenetik, Praxis Erfurt, Erfurt, Germany.*

In the process of our optical genome mapping verification study, 7 cases with an apparently balanced translocation were examined to gain deeper insight into the translocation breakpoints. In 3 cases, we were able to narrow down the translocation breakpoints to a relevant gene by optical genome mapping thus identifying the cause of the patients' symptoms. As an example we present a 23 years old woman with a learning disability, who is carrier of a translocation t(3;20), analyzed by chromosome- and microarray analysis. In the family, the mother and 2 half siblings are carriers of the translocation t(3;20) and are also affected with learning disability. Using Bionano Genomics technology, we were able to pin down the translocation breakpoints to the following genes: *AC096887.1* (breakpoint on chromosome 3) and *CHD6* (breakpoint on chromosome 20; OMIM *616114). This case shows that optical genome mapping is very helpful and well suited to detect translocations and to characterize balanced translocation breakpoints.

J. Schiller: None. **K. Bilska:** None. **U. Heinrich:** None. **E. Krimmel:** None. **S. Demuth:** None. **I. Rost:** None.

P14.024.A Optical genome mapping: a cytogenetic revolution

Valeria Orlando[1], *Silvia Genovese*[1], *Laura Ciocca*[1], *Viola Alesi*[1], *Sara Loddo*[1], *Silvia Di Tommaso*[1], *Marina Trivisano*[2], *Maria Lisa Dentici*[3], *Alessandro Ferretti*[2], *Antonio Novelli*[1]

[1]*1- Laboratory of Medical Genetics, Translational Cytogenomics Research Unit, Bambino Gesù Children Hospital, Roma, Italy,* [2]*2- Rare and Complex Epilepsy Unit, Department of Neuroscience, Bambino Gesù Children Hospital, Roma, Italy,* [3]*3- Medical Genetics Unit, Academic Department of Pediatrics, Bambino Gesù Children Hospital, Roma, Italy.*

Balanced translocations are chromosome structural rearrangements relatively common in human population. A fine characterization of the rearrangement is crucial for the identification of gene disruption at breakpoints and for the evaluation of the pathological outcome. Karyotype, FISH and CMA can provide important genomic information but cannot define rearrangements at a proper resolution.We describe a 6-years-old female with normal development until epilepsy onset at 16 month with a febrile seizure. Then she experienced drug resistant generalized or focal to generalized seizures often in clusters and cognitive decline. Diagnosis of Dravet Syndrome was made. At last follow up she showed drug resistant epilepsy with daily seizures, cognitive, motor, and severe language deficits and behavioural disturbances. NGS analysis did not reveal pathogenic variants in genes associated with epileptic encephalopathies. Karyotype analysis showed a balanced translocation involving chromosomes 2 and 18 with breakpoints at 2q24.3 and 18q21.1. Microarray analysis detected a 181 Kb de novo microdeletion at 2q24.3, involving SCN2A gene.Optical Genome Mapping(OGM) was used to characterize the rearrangement and to verify the correlation between the 2q24.3 microdeletion and the translocation breakpoint. OGM confirmed the SCN2A microdeletion but showed a complex rearrangement involving four different breakpoints. In particular, a paracentric inversion at 2q24.3 was shown to disrupt SCN1A gene, associated to Dravet syndrome. Our patient's severe phenotype is due to the concomitant loss of function of SCN1A and SCN2A.This new technology allowed a proper characterization of the rearrangement, defining a better molecular diagnosis that leads to precise treatments and clinical outcome.

V. Orlando: None. **S. Genovese:** None. **L. Ciocca:** None. **V. Alesi:** None. **S. Loddo:** None. **S. Di Tommaso:** None. **M. Trivisano:** None. **M. Dentici:** None. **A. Ferretti:** None. **A. Novelli:** None.

P14.025.B A novel case of a girl with partial trisomy 12q24.21q24.33 and review of the literature

Lautaro Plaza-Benhumea[1], *Monica Martin-DeSaro*[2], *Olga Messina-Baas*[3], *Sergio Cuevas-Covarrubias*[4]

[1]*Hospital Perinatal, Monica Pretelini, EdoMex, Mexico,* [2]*Pediatrics Department, Hospital Materno Infantil ISSEMyM, EdoMex, Mexico,* [3]*Hospital General de Mexico, CDMX, Mexico,* [4]*Hospital General de*

Mexico, Universidad Nacional Autonoma de Mexico, CDMX, Mexico.

Abnormalities of chromosome 12, specially 12p, can occur commonly resulting in well-known phenotypes, but trisomy 12q is rarely reported. The majority of cases had duplication involving the region 12q24/qter due to segregation of a maternal balanced translocation. Other rearrangements involved translocations with chromosomes 2, 4, 5, 9, 11, 17, 18, 21, mosaicism or pericentric inversion. Trisomy 12q can be characterized by a clinically recognizable syndrome including craniofacial dysmorphy, growth failure, occasional brain malformations, abnormalities of the extremities, skeletal and thoracic malformations, cardiovascular defects, anogenital abnormalities like cryptorchidism, psychomotor delay and ontellectual disability The microarray assay exhibited an approximately 19.35Mb gain of the long arm of chromosome 12 at 12q24.21q24.33 (Fig. 3). The mother´s FISH showed ish ins(21;12)(p11.2;q24.21q24.33)(RP11-946G22+). We report the case of a girl with partial trisomy 12q24.21q24.33.

L. Plaza-Benhumea: None. **M. Martin-DeSaro:** None. **O. Messina-Baas:** None. **S. Cuevas-Covarrubias:** None.

P14.026.C Expanding phenotype in a patient with partial trisomy 13q/monosomy 3p resulting from a paternal reciprocal 3p;13q translocation

Monica Martin-DeSaro[1], *Lautaro Plaza-Benhumea*[2], *Luz Gonzalez-Huerta*[3], *Olga Messina-Baas*[4], *Sergio Cuevas-Covarrubias*[5]

[1]*Medical Genetics Department, Hospital Materno Infantil ISSEMyM, EdoMex, Mexico,* [2]*dHospital Perinatal, Monica Pretelini, EdoMex, Mexico,* [3]*Hospital General de México, EdoMex, Mexico,* [4]*Hospital General de Mexico, CDCMX, Mexico,* [5]*Hospital General de Mexico, Universidad Nacional Autonoma de Mexico, CDMX, Mexico.*

Individuals with 3p deletion present a great clinical variability. Apparently, a 1.5 Mb terminal deletion, including *CRBN* and *CNTN4* genes, is sufficient to cause this syndrome. Partial trisomy 13q is an uncommon chromosomal abnormality with a variable phenotypic expression, but in most cases patients have a phenotype resembling complete trisomy 13. The aim of the present study is to describe a Mexican 9-old-months male with 3pdel/13qdup and a novel clinical finding. He presented facial dysmorphism and multiple congenital alterations. Echocardiogram reported cardiac insufficiency with hypertrophic cardiomyopathy and pulmonar hypertension, not previously reported. Karyotype from the father was 46,XY,t(3;13). Microarray assay of the proband exhibited an approximately 2.6Mb loss at terminal 3p26.3 and a 27.7Mb gain of the long arm of terminal chromosome 13 at q31.1q34. The proband suffered a chromosomal unbalance with a partial trisomic component 13q31.1-q34 and a monosomic component 3p26.3 from paternal origin. Microarray assay of both parents were normal. Although clinical spectrum is too high in this chromosomal aberration, the proband showed a cardiomyopathy not previously reported. This data enriches the spectrum of clinical manifestations in 3pdel/3qdel chromosomopathy.

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P14.027.D In depth evaluation of a 9p tetrasomy

Maximilian Radtke, Mareike Mertens, ***Susanna Schubert***

University of Leipzig Hospitals and Clinics Leipzig, Leipzig, Germany.

Introduction: We present a rare case of hypertrophy of the choroid plexus with chromosome 9p triplication. Chromosome 9p triplication is an abnormality with two extra copies of genetic material on the short arm (p) of chromosome 9. The symptoms and the severity of the condition depend on the size and location of the triplication and the genes that are involved. The general symptoms include growth retardation, recurrent joint dislocations, scoliosis, developmental delay, intellectual disability, behavioral problems and distinctive facial features.

Materials and Methods: We report a case of a 1-year old male patient presenting in our genetic counselling unit with speech and motor developmental delay, hydrocephalus and hypertrophy of the choroid plexus with consecutive ventriculomegaly. The family history is unremarkable. We performed exome-sequencing with subsequent karyotyping and FISH-analysis. For specific delineation of the chromosome 9 haplotype, we conducted a NGS-based allele-fraction analysis.

Results: The NGS-based CNV-analysis revealed a 40 Mb triplication (CN4) on chromosome 9, region 9p:31023-40232529. Karyotyping (GTG- and C-banding) and FISH-analysis (fluorescence in situ hybridisation) with a CEP 9 probe (9p11-q11 Alpha Satellite DNA) revealed an additional marker chromosome, which was identified as an additional isodicentric chromosome 9p.

Conclusion: The karyotyping, FISH- and bioinformatic analysis revealed that the initial NGS-based diagnostic of a 9p triplication turned out to be a complete tetrasomy 9p with an additional isodicentric chromosome. Our genetic analysis therefore supports the importance of gene dosage measurement with NGS-based CNV analysis, Karyotyping, and FISH analysis for identifying causes of choroid plexus hypertrophy.

M. Radtke: None. **M. Mertens:** None. **S. Schubert:** None.

P14.028.A Case report: a reciprocal translocation between chromosomes 4 and 12 at a 14 years old boy

Sandra Grigore[1], *Doina Guzun*[1,2], *Florin R. Nitu*[1]

[1]*Sante Clinic, Bucharest, Romania,* [2]*Fundeni Clinical Institute, Bucharest, Romania.*

Introduction: Reciprocal translocations occur when part of one chromosome is exchanged with another part of another chromosome. Translocations can disrupt functional parts of the genome and have implications for protein production with phenotypic consequences. Reciprocal translocations are one of the most common structural chromosome reorganizations in humans, with an incidence of approximately 0.14% in newborn.

Materials and Methods: We present a case of a 14 years old boy with obesity, puberty delay and cryptorchidism.

Results: High resolution chromosome analysis revealed a reciprocal translocation between chromosomes 4 and 12 with ISCN formula: 46,XY,t(4;12)(q33;q22).

Conclusion: We didn't find another case reported with this transocation. We believe that the translocation breakpoints disrupt an essential gene, and the gene is inactivated and behaves as a point mutation, which could explain the phenotype. It would be useful to perform molecular analysis for establishing the affected gene.

S. Grigore: None. **D. Guzun:** None. **F.R. Nitu:** None.

P14.029.B Increased methylation of genes responsible for fetal-maternal interaction in chorionic villi of miscarriages with trisomy 16

Stanislav A. Vasilyev[1,2], *Ekaterina N. Tolmacheva*[1], *Oksana Yu. Vasilyeva*[1], *Tatiana V. Nikitina*[1], *Elena A. Sazhenova*[1], *Anton V.*

Markov[1], Ekaterina S. Serdyukova[2], Viktoria V. Demeneva[1], Igor N. Lebedev[1]

[1]Scientific Research Institute of Medical Genetics, Tomsk National Research Medical Center, Tomsk, Russian Federation, [2]National Research Tomsk State University, Tomsk, Russian Federation.

Aneuploidy is the leading cause of early human embryonic death with trisomy 16 being the most frequent. Moreover, trisomy 16 is frequently found confined to the placenta, with a chromosomally normal fetus. According to our results obtained using Infinium HumanMethylation27 BeadChip (Illumina), miscarriages with trisomy 16 showed significant hypermethylation in promoters of 90 genes (deltaB>0.15). Among them, the genes of secreted proteins were enriched (29 genes, $p = 5.8$ E-8), and 10 more genes encoded receptors. This makes it relevant to study the effects of trisomy 16 on epigenetic regulation of genes responsible for trophoblast differentiation and fetal-maternal interaction. DNA methylation level of promoters of five genes (ANKRD53, GATA3, CALCB, TRPV6, and SCL13A4) was analyzed in detail using targeted bisulfite massive parallel sequencing in the chorionic villus trophoblast of 22 miscarriages with trisomy 16 and 10 induced abortions.

Miscarriages with trisomy 16 had elevated DNA methylation level in the promoters of all studied genes compared to induced abortions (from 1 to 45 differentially methylated CpG-sites per gene, 6-38 % of all analyzed CpG-sites, $p < 0.05$). Specific differentially methylated CpG-sites were identified in transcription factor binding sites. Obtained results indicate that epigenetic abnormalities, potentially affecting the embryonic genome stability and regulation of the signal interaction between aneuploid embryo and mother, can be a potential cause of pregnancy termination in the presence of aneuploidy in extraembryonic tissues.

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P14.030.C Uterine leiomyomas with an apparently normal karyotype comprise cryptic heteroploid cell subpopulations

***Alla S. Koltsova**, Anna A. Pendina, Olga A. Efimova, Olga G. Chiryaeva, Natalia Yu Shved, Maria I. Yarmolinskaya, Nikolai I. Polenov, Vladislava V. Kunitsa, Tatiana G. Tral, Gulrukhsor Kh Tolibova, Vladislav S. Baranov*

D.O. Ott Research Institute of Obstetrics, Gynecology and Reproductology, St. Petersburg, Russian Federation.

Cytogenetic analysis of uterine leiomyoma (UL) cell cultures reveals clonal chromosome abnormalities in 60% of ULs. The latter are believed to occur secondarily during tumorigenesis. We aimed to check whether ULs with an apparently normal karyotype comprise "hidden" cell subpopulations with numerical chromosome abnormalities (heteroploid cells). Using interphase FISH with centromeric DNA probes (Vysis CEP 7 (D7Z1) SpectrumGreen and Vysis CEP 16 (D16Z3) SpectrumOrange, AbbottLaboratories), we analyzed the copy number of chromosomes 7 and 16 in nine karyotypically normal ULs. Chromosome copy number was screened in 1000 cultured and 1000 non-cultured cells of each UL. All of the ULs included both normal disomic cells representing a predominant subpopulation and heteroploid cells reaching a maximum frequency of 21.6% (mean 9.8%) in vivo and 11.5% (mean 6.1%) in vitro. The spectrum of heteroploid cells was similar in vivo and in vitro and mostly consisted of monosomic and tetrasomic cells. However, their frequencies in the cultured samples differed from those in the uncultured ones: while the monosomic cells decreased in number (Wilcoxon signed-rank test, $p = 0.0195$), the tetrasomic cells became more numerous (Wilcoxon signed-rank test, $p = 0.0078$). Our results suggest that ULs with an apparently normal karyotype revealed in vitro consist of both karyotypically normal and heteroploid cells. Different frequencies of heteroploid cells in vivo and in vitro suggest their regulation by tumor microenvironment and point towards their significance for UL pathogenesis. Supported by RSF №19-15-00108.

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P14.031.D Investigation of a de novo complex intrachromosomal X chromosome rearrangement in a girl with primary amenorrhea

Voula Velissariou

BIOIATRIKI HEALTH GROUP, ATHENS, Greece.

A 16-year-old female with primary amenorrhea, hypoplastic womb, fully developed secondary female characteristics, normal height and learning difficulties was referred for CMA studies. CMA analysis (Cytoscan 750K, Affymetrix) revealed a female profile with numerous deletions and duplications affecting one of the two X chromosomes. In total, fifteen different clinically significant CNVs were identified. Of these, the largest is an 81 Mb deletion in the long arm at Xq13.2q28 containing 370 OMIM genes and two deletions in the short arm at Xp22.2p22.11 and Xp11.3p11.23, 11.2Mb and 3.8Mb in size, containing 56 and 46 OMIM genes, respectively. The presence of XIST was confirmed with locus specific FISH. Furthermore, X-Inactivation studies using the **Androgen Receptor** (AR) gene, showed completely skewed inactivation pattern. High resolution GTG-banding, revealed a 46, X,der(X) karyotype in the proband, while parental studies were normal. Molecular karyotyping by Multi-color FISH (Metasystems) ruled out inter-chromosomal rearrangements, showing that the abnormal sex chromosome is solely composed of genomic material deriving from chromosome X. Combined with the CMA analysis revealing multiple adjacent duplication/deletion regions in the highly remodeled p-arm of the derivative X-chromosome, our data suggest the involvement of serial fork stalling and template switching (FoSTeS), or microhomology-mediated break-induced replication (MMBIR) events, leading to Chromoanasynthesis in the Lyonized aberrant chromosome. The phenotypic outcome of this rare sex chromosome rearrangement may be attributed to combined dosage effects of genes that escape X-inactivation. [SG1]This can be excluded

V. Velissariou: None.

P15 New Technologies and Approaches

P15.001.A A complex DMD gene mutation of a carrier revealed by linked read WGS

***Maria Elena Onore**[1], Annalaura Torella[1], Francesco Musacchia[2], Francesca Del Vecchio Blanco[1], Paola D'Ambrosio[1], Mariateresa Zanobio[1], Giulio Piluso[1], Vincenzo Nigro[1,2]*

[1]Università degli Studi della Campania Luigi Vanvitelli, Naples, Italy, [2]Tigem- Telethon Institute of Genetics and Medicine, Pozzuoli, Italy.

Today, the use of NGS gene panels, WES or WGS, has changed our approach to the diagnostic process. However, NGS analysis could be ineffective in identifying large and complex genomic rearrangements. In the effort to overcome this limitation, we have applied a new 10x linked-read sequencing technology that combines single-molecule barcode with short-read, to solve NGS-negative patients. First, we were able to distinguish similar genes, as *SMN1* and *SMN2* and identify structural variants in genes with similar pseudogenes such as *PKD1* or *PKD2*. We also performed WGS on two trios with rare diseases who were WES-negative. Particularly interesting is the use to define the large rearrangements in the *DMD* gene which are the most common cause of dystrophinopathies including Duchenne (DMD) and Becker (BMD) muscular dystrophy. Here, we show the case of a DMD carrier with an unsolved genetic state. A deletion of exons 16-29 in DMD gene was responsible for BMD phenotype in male of her family. MLPA and array-CGH analysis showed a normal dosage of these exons and an increased dosage of flanking exons 1-15 and 30-34. The WGS 10x was able to phase both X chromosomes, showing the deletion of exons 16-29 on one allele and the duplication of exons 1-34 on the second one in the *DMD* gene. In conclusion, our results demonstrate that 10x linked-read technology can be a useful tool for improving our understanding of unsolved genetic conditions in a very feasible way.

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P15.002.B The clinical utility of optical genome mapping for the assessment of genomic aberrations in acute lymphoblastic leukemia

Jonathan Lukas Lühmann[1], *Marie Stelter*[1], *Marie Wolter*[1], *Josephine Kater*[1], *Jana Lentes*[1], *Anke Katharina Bergmann*[1], *Max Schieck*[1], *Gudrun Göhring*[1], *Anja Möricke*[2], *Gunnar Cario*[2], *Martin Schrappe*[2], *Brigitte Schlegelberger*[1], *Martin Stanulla*[3], *Doris Steinemann*[1]

[1]Department of Human Genetics, Hannover Medical School, Hannover, Germany, [2]Department of Pediatrics I, ALL-BFM Study Group, Christian-Albrechts University Kiel and University Medical Center Schleswig-Holstein, Kiel, Germany, [3]Pediatric Hematology and Oncology, Hannover Medical School, Hannover, Germany.

Introduction: Acute lymphoblastic leukemia (ALL) is the most prevalent type of cancer occurring in children. Nowadays, 85-90% of patients with ALL can reach a long-term cure; yet, ALL does reoccur in about 15-20% of all patients and can be cured in just 30%-50% of the relapsed cases. ALL is molecularly characterized by an increasing number of structural genomic aberrations that strongly correlates with prognostic and clinical outcomes. Usually, a combination of cyto- and molecular genetic methods (karyotyping, array-CGH, FISH, RT-PCR, RNA-seq) is needed to identify all the underlying genomic aberrations present in ALL. This research aims to investigate the feasibility of optical genome mapping (OGM), a DNA-based method, to detect structural variants in an all-in-one approach.

Methods: As proof of principle, twelve pediatric B-cell precursor ALL samples from ALL-BFM-2000 and AIEOP-BFM-ALL-2017 were analyzed by means of OGM using the Saphyr system (Bionano Genomics). Intensive validation of the results was performed by comparing OGM data to existing data from routine diagnostic workflows. Result: All structural alterations among translocations (e.g., t(1;19), dic(9;12)), aneuploidies (e.g., -7, -11, high hyperdiploidy), and copy number variations (e.g., *IKZF1*^{plus} profile) known from established techniques were detected by OGM as well. Moreover, OGM revealed a more complex structure of a known translocation t(12;21) and detected additional alterations, among those a small deletion in *SETD2* as well as a stratification relevant gene fusion of *JAK2*/*NPAT* in an archived sample.

Conclusion: This pilot study demonstrates that OGM has the potential to detect stratification-relevant markers in an all-in-one approach in ALL.

J.L. Lühmann: None. **M. Stelter:** None. **M. Wolter:** None. **J. Kater:** None. **J. Lentes:** None. **A.K. Bergmann:** None. **M. Schieck:** None. **G. Göhring:** None. **A. Möricke:** None. **G. Cario:** None. **M. Schrappe:** None. **B. Schlegelberger:** None. **M. Stanulla:** None. **D. Steinemann:** None.

P15.003.C ACACIA: A Customized Array Cgh for solving unsolvable genetic dIseAses

Annalaura Torella[1,2], *Francesca Del Vecchio Blanco*[1], *Giancarlo Blasio*[1], *Mariateresa Zanobio*[1], *Roberta Zeuli*[1], *Maria Elena Onore*[1], *Francesca Romano*[1], *Cristina Peduto*[1], *Francesco Piluso*[1], *Michela Napoli*[1], *Esther Picillo*[1], *Giovanni Vicidomini*[1], *Floriana Secondulfo*[1], *Paola D'Ambrosio*[1], *Giulio Piluso*[1], *Vincenzo Nigro*[1,2]

[1]Università degli Studi della Campania "Luigi Vanvitelli", Napoli, Italy, [2]Tigem-Telethon Institute of Genetics and Medicine, Pozzuoli, Italy.

The introduction of whole exome sequencing has allowed a successful diagnosis of genetically heterogeneous disorders inducing enormous optimism for the future of rare disease diagnosis. However, about 50% of patients do not receive a molecular diagnosis, making it crucial to develop and implement strategies for understanding all mechanisms underlying rare diseases. To date, the trio analysis of WES is powerful in detecting qualitative DNA changes, but nearly blind in recognizing small quantitative changes like intragenic deletions/duplications. Although many algorithms have been developed to derive quantitative information from WES data, these remain inaccurate, needing further exon-by-exon validation. Therefore, we set out to develop a new high-resolution quantitative test, designing an exome-based array CGH (ACACIA) with a full single-exon coverage. The design provides probes covering 7,835 genes: all known human disease genes are included plus 2,599 candidate genes with lower- than-expected mutations. ACACIA was developed using the Agilent SurePrint G3 Custom CGH Microarray, 1x1M technology. Under the Telethon Undiagnosed Diseases Program (TUDP), a large cohort of over 600 rare disease patients is already available. Of these, approximately 50% are still without disease causing mutations or with only one putative mutation in gene of known recessive disorders. Preliminary validation experiments confirmed that ACACIA is able to identify small intragenic copy number mutations (CNMs), even when a single exon is involved. Unsolved pediatric cases from the TUDP project will be analyzed using ACACIA. We are confident that an additional percentage of cases can be solved with this innovative approach.

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P15.004.D Automated variant classification workflows maintain quality standards, support standardisation and reduce turn-around times in a rare disease laboratory

Helen Savage

Congenica Ltd, Hinxton, United Kingdom.

As demand for genomic testing increases, variant analysts encounter increasingly complex patients in addition to their routine cases. While increased access to testing should be celebrated, the use of first-line exome and genome testing makes variant interpretation a key bottleneck, as highly skilled analysts are not experts on all genes/disorders encountered.

A timely diagnosis has a huge impact on patients and families; providing answers to questions such as "why is my child different?", informing reproductive choice, providing access to support, information about prognosis and early treatment to improve patient outcomes. However, the increased effort needed to familiarise analysts with novel genes and variants increases turnaround times and may lead to delays in reporting. With a finite workforce laboratories may face a lack of resources for analysis of complex cases, leading to an extended diagnostic odyssey for patients and their families. This issue was confirmed in a survey of Clinical Laboratories, which identified that 71% are at, or approaching, capacity.

Automation is commonplace in the diagnostic laboratory; from liquid-handling robotics, to automated bioinformatics pipelines processing large volumes of data. As unsupported manual interpretation is not a scalable solution, is it time to embrace semi-automation of variant interpretation, to provide the genomics workforce with more time to diagnose the most complex cases, so that no patient is left behind?

Here we present the case for automating analysis of cases, to rapidly generate high-quality, relevant results supporting the diagnosis of patients with rare disease, without compromising the diagnostic yield each analysis.

H. Savage: A. Employment (full or part-time); Significant; Congenica Ltd.

P15.005.A Automation of NGS library preparation for low input, degraded samples

Li Liu

Beckman Coulter Life Sciences, Indianapolis, IN, USA.

Introduction: Over the years, Next Generation Sequencing (NGS) workflows have become more and more complex, and they include a variety of sample types such as Formalin Fixed Paraffin Embedded (FFPE) samples and Cell-free DNA (cfDNA). Despite their relevance in disease-oriented research, sequencing FFPE and cfDNA samples remain challenging due to their low DNA quality and quantity. The creation of libraries for NGS itself is a tedious process further challenged by difficult sample types. Automation of library preparation can relieve the burden, but not all liquid handlers are the same, especially when it comes to minimizing errors.

Methods: In this poster, we present automated performance of the IDT xGen Prism DNA library preparation kit on the new Biomek NGeniuS liquid handler. With 4 cfDNA samples (10 ng each) we generated libraries following a modular automated workflow that minimized hands-on time and manual interactions such as reagent aliquoting. We sequenced the samples on an Illumina NextSeq 550 sequencer and mapped the sequenced reads against human reference genome using Burrows-Wheeler Aligner.

Results: All the libraries passed the vendor recommended quality assessments and we observed no signs of DNA or sample loss. After sequencing, >99.5% of the reads aligned with the reference. Percentage of singletons were <0.23%. In terms of percentage reads aligned, automated libraries showed less variability between replicates than manually prepared libraries.

Conclusions: The results indicated that the automated workflow is successful at preparing NGS libraries from low DNA quantity challenging samples such as cfDNA.

L. Liu: A. Employment (full or part-time); Significant; Beckman Coulter life Sciences.

P15.006.B Automation of NGS library preparation for cancer panels

***Kristina Lu**, Zachary Smith*

Beckman Coulter Life Sciences, Indianapolis, IN, USA.

Introduction: Next Generation Sequencing (NGS) has become the gold standard in oncology research. As the sequencing cost (e.g., instrumentation and sequencing chemistry) decreases and the sequencing data storage and analytics capacity increases, efficient library preparation methods are becoming increasingly important for the diagnosis, prognosis and treatment of cancer. Parallel processing of samples through automation can help to rapidly create NGS libraries with minimal hands-on time. However, the quality of the created library is also important for the downstream analysis.

Methods: In this poster, we evaluate the automation of the AmpliSeq for Illumina Cancer HotSpot Panel v2 protocol on the new Biomek NGeniuS. We loaded the liquid handler with 6 samples at the input concentration of 10 ng. We sequenced the libraries on an Illumina NextSeq 550 sequencer and analyzed the data using BaseSpace.

Results: We created libraries using the standard workflow and all libraries passed the quality thresholds. Our sequencing results showed that >97% of reads aligned with the target with uniform coverage. We were able to identify all gene variants expected from the panel at high allele frequency.

Conclusions: Our results show that, apart from time savings, automation can also create high-quality NGS libraries.

K. Lu: A. Employment (full or part-time); Significant; Beckman Coulter life Sciences. **Z. Smith:** A. Employment (full or part-time); Significant; Beckman Coulter life Sciences.

P15.007.C Automation of NGS library preparation for hybrid capture

Zachary Smith

Beckman Coulter Life Sciences, Indianapolis, IN, USA.

Introduction: Next Generation Sequencing (NGS) has revolutionized Biology due to its high throughput, scalability and speed. As a result, scientists are able to study biological systems at a depth never before possible, to find answers to complex biological questions. Despite these advancements, the creation of NGS libraries is a tedious process. Protocols such as hybrid capture can take several days to complete and require multiple manual interactions and pipetting.

Methods: To overcome these challenges, we automated Agilent's SureSelect XT kit combined with the Human Exon V6 panel, which has a two-day long workflow on the new Biomek NGeniuS liquid handler. We started with four samples of Coriell

DNA, at input concentration of 200 ng. After shearing, we placed the samples and kit reagents on the liquid handler, in their original reagent vials. We prepared the libraries in the automated liquid handler, following vendor recommended safe stop points. We sequenced the libraries on an Illumina NextSeq 550 sequencer and analyzed the data using BaseSpace.

Results: All libraries passed the vendor recommended QC threshold. Our sequencing data showed that the automated libraries covered >93% targeted regions and >97% of the reads aligned with the reference.

Conclusions: Our automation approach successfully created sequencing-ready libraries while minimizing manual pipetting, handling errors and manual touchpoints.

Z. Smith: A. Employment (full or part-time); Significant; Beckman Coulter life Sciences.

P15.008.D Comprehensive carrier screening strategy for challenging genomic conditions

Evrim Unsal, *Suleyman Aktuna, Leyla Ozer, Merve Polat, Volkan Baltaci*

Yuksek Ihtisas University, Ankara, Turkey.

Introduction: Universal carrier screening test is a widespread approach for determining couples with increased risk of having an affected child due to ethnicity or consanguineous marriage. Hotspot screening for common mutations has been replaced by NGS-based Expanded Carrier Screening (ECS) covering 100-400 diseases. Recent idea is that specificity/sensitivity and uniform coverage are more important than the number of genes involved. In this study, the validation data of 64 patients with carrier screening panel targeting coding regions of 420 genes are presented.

Materials and Methods: A multiplex sequencing panel targeting 100% of coding bases plus flanking regions for 420 genes was created with Ion AmpliSeq™ Designer and data analysis was perfomed using Carrier Reporter Software. 32 clinical samples with known variants including pseudogene mutations and CNVs were tested. 18 genes difficult to analyze due to presence of pseudogenes, gene conversions and paralogues were selected for the study including GBA, HBA1/HBA2, CYP21A2, CYP11B1, SMN1/SMN2, VWF. CFTR and DMD genes are added for CNV information.

Results: Results revealed that 27 of 35 previously detected variants are called by the analysis algorithm. 8 false negatives were detected in 6 genes (CYP21A2, GLA, GBA, CYP11B1, HBA1/HBA2, ITGB3, VWF). Additionally, potential false positive calls were detected, majority of which lies in gene coversion regions (ABCC6, CBS, CYP21A2).

Conclusions: These results suggest that a correction algorithm for gene conversion is required. After the initial analysis, special algorithms were adopted enabling detection of all variants in 8 false negative samples making the test 100% sensitive for challenging variants.

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P15.009.A The first Saudi baby with classic homocystinuria diagnosed by universal newborn screening

TALAL SALEH ALANZI, *Sarar Mohamed, Fahad AlHarbi, Joharah AlFaifi*

Prince Sultan Military Medical City, Riyadh, Saudi Arabia.

Classic homocystinuria (CH) is an inborn error of metabolism caused by cystathionine beta-synthase enzyme deficiency. Affected patients present with intellectual disability and other comorbidities. If diagnosed early in infancy and started treatment, inevitable complications can be prevented. Newborn screening (NBS) uses tandem mass-spectroscopy (MSMS) to measure the amino acid levels. In CH, the first-tier screening test is the measurement of methionine by MSMS. If methionine remained elevated in the recall sample, plasma level for homocysteine is performed. A newborn infant underwent routine NBS in our institute that showed elevated methionine in the first and the recall sample. Thereafter, total serum homocysteine was found to be elevated, consistent with the diagnosis of CH. An early medical and dietary management was commenced for this first Saudi baby diagnosed with homocystinuria by universal NBS. This report demonstrates that NBS for CH is feasible and effective in preventing the disease burden.

T.S. Alanzi: None.

P15.010.B Optimized shallow whole-genome sequencing for large CNV detection in rare genetic disorders

Anett Marais, *Krishna Kumar Kandaswamy, Antonio Romito, Natalia Ordonez, Dan Diego Alvarez, Katja Bruesehafer, Volkmar Weckesser, Peter Bauer, Jonas Marcello*

Centogene AG, Rostock, Germany.

Large copy number variations (CNVs) represent a significant fraction of genomic alteration in human disease. Next Generation Sequencing (NGS) methods are increasingly used for CNV detection, slowly replacing chromosomal microarray analysis (CMA). Low covered genomic regions and representation bias can be reduced by NGS based CNV analysis without increasing error rates or loosing robustness. Benefits of shallow WGS (sWGS) over CMA were evaluated in a detailed method comparison and verified on birth defects, but the challenge of affordable rapid settings for large scale clinical testing remained largely unsolved.

We demonstrate the feasibility of sWGS implementation in routine diagnostics of rare genetic disorders based on a systematic comparative analysis to CMA and introduce CentoLCV™, an end-to-end sWGS pipeline for large scale detection of clinically relevant copy number alteration. The sensitivity is significantly higher compared to CMA with an average of 147 CNVs per sWGS sample. We find 13x higher CNV detection capabilities for 10-20kb sized CNVs with an overall increase of 6x over the whole size range, while others report up to 20x increase with a majority of detections in the range of 1-10kb, the most prevalent range in standard samples. Our diagnostic rate is at 22.4%, resulting in a clear pathogenic finding in 7.1 % cases, in line with previous studies. Furthermore, we detect chromothripsis and triploid events.

Altogether, we confirm sWGS as an excellent tool to perform CNV detection in large scale diagnostics and suggest a rapid replacement of CMA analysis in favor of resolution, throughput and affordability.

A. Marais: A. Employment (full or part-time); Modest; Centogene AG. **K.K. Kandaswamy:** A. Employment (full or part-time); Modest; Centogene AG. **A. Romito:** A. Employment (full or part-time); Modest; Centogene AG. **N. Ordonez:** A. Employment (full or part-time); Modest; Centogene AG. **D.D. Alvarez:** A. Employment (full or part-time); Modest; Centogene AG. **K. Bruesehafer:** A. Employment (full or part-time); Modest; Centogene AG. **V. Weckesser:** A. Employment (full or part-time); Modest; Centogene AG. **P. Bauer:** A. Employment (full or part-time); Modest;

Centogene AG. **J. Marcello:** A. Employment (full or part-time); Modest; Centogene AG.

P15.012.D Implementation barriers of dynamic consent in clinical genetics

Tita Alissa Bach, *Sharmini Alagaratnam, Serena Elizabeth Marshall*

DNV GL, Oslo, Norway.

Introduction: The reach and relevance of clinical genetics to complement the increased prevalence of personalised diagnostic and therapeutic decision making for patients is growing. To accompany this, there are demands for technologies and processes that support informed patient decision making. Dynamic consent is an approach that can facilitate two-way communication, setting and modifying of consent preferences by patients over time and meet the needs of clinical genetics environments. Despite these benefits, implementation to date is limited.

Methods: A literature review and semi-structured interviews with experts, supported by a survey of clinical genetics professionals, were employed to identify and map the barriers to implementation of dynamic consent in clinical genetics.

Results: The findings revealed six categories of barriers and sub-barriers: 1. ethical barriers (ensuring trust, autonomy versus information overload, sharing data, and revoking previously consented data), 2. legal and regulatory barriers (regulation, use of data, and the GDPR), 3. knowledge and competence barriers (consent comprehension, and variable user backgrounds), 4. financial barriers (investment versus gain), 5. cultural and organisational barriers (stakeholder engagement and collaboration, and cultural shift), 6. technological barriers (security, traceability and transparency, and interoperability).

Conclusion: In addition to the benefits of dynamic consent in clinical genetics environments, it also has the potential to support paradigm shifts for medicine in other specialties. As more use cases develop where dynamic consent approaches can be applied, the barriers identified pinpoint several focus areas that should be considered prior to and as developments of dynamic consent solutions are underway.

T. Bach: None. **S. Alagaratnam:** None. **S. Marshall:** None.

P15.013.A Polyvalent CD8A CSR messenger RNA encoded to generate a modified transcription factor IIIA polypeptide intended to seek out, bind to and neutralize the COVID-19 genome, this modified human CD8A CSR mRNA additionally carrying nucleotide coding to generate the COVID-19 B1.1.7 spike protein to be mounted on the surface of the cell, to act as a vaccine

Lane B. Scheiber II, *Lane B. Scheiber*

Osteoporosis & Arthritis Center, Richmond, VA, USA.

Introduction: The coronavirus pandemic has demonstrated a need to strike directly at viral genomes inside host cells.

Materials and Methods: Utilizing teachings of human genetics, the CD8A CSR messenger ribonucleic acid (mRNA) was re-coded to carry both a cure for COVID-19 and an updated vaccine.

Results: Following 5′ leader end of CD8A mRNA, is attached nucleotide coding for a modified human TFIIIA polypeptide (TI9606) designed to target the poly uracil tail of negative-sense coronavirus's genome. Utilizing published amino acid-nucleotide binding algorithm, we altered zinc fingers 1-6 of native human TFIIIA polypeptide to cause the protein to seek out and bind to the 33 uracil nucleotide tail of the negative-sense coronavirus genome. Fixing a 365 amino acid polypeptide to coronavirus genome interferes with replication of the virus, halting the pathogen's infectious nature. Following a UAA 'AND' command, we added nucleotide coding for the 'S' protein associated with B1.1.7 variant, to include N501Y and E484K variants to the Wuhan-Hu-1 coronavirus, followed by a second UAA, then followed by 3′ terminal end of CD8A mRNA and a poly adenine tail.

Conclusion: We designed a 7,029 nucleotide medically therapeutic mRNA to generate two polypeptides. First protein, a modified TFIIIA polypeptide to bind to the COVID-19 genome; second protein to be mounted on the surface of the cell alerting the immune system to B1.1.7 spike protein. Administration via nanomicelle technology.

Polyvalent mRNA to both neutralize Coronavirus genome and function as a COVID-19 B1.1.7 vaccine



L.B. Scheiber II: None. **L.B. Scheiber:** None.

P15.014.B Inhibition of *MAD2L2* and *NUDT16L1* impacts homology directed repair in CRISPR-Cas9 gene editing

Arina A. Anuchina, *Milyausha I. Zaynitdinova, Anna G. Demchenko, Svetlana A. Smirnikhina, Alexander V. Lavrov*

Research Centre for Medical Genetics(RCMG), Moscow, Russian Federation.

Introduction: Enhancing gene editing efficacy through impacting repair mechanisms is the widespread approach when using CRISPR-Cas9 system. After introducing double-strand break (DSB) several repair mechanisms can act. Two major are non-homologous end joining (NHEJ) and homology directed repair (HDR). NHEJ is the major, however, error-prone way, while HDR provides correct donor-template repair. To enhance HDR efficacy and inhibit NHEJ we use knockdown of *MAD2L2*, one of NHEJ participants, and overexpression of *NUDT16L1*, that inhibit the main NHEJ factor action.

Materials and methods: Experiments were performed on HEK293T cell line. CRISPR-Cas9 plasmid with *spCas9(1.1)* gene and sgRNA cassette targeted to the mutated GFP sequence was co-transfected with peGFPmut plasmid with *eGFPmut* gene under CMV-promoter. ssODN with 143bp of wild-type GFP sequence was used as repair template for HDR. Plasmid with *NUDT16L1* CDS under CMV promoter was used for *NUDT16L1* overexpression. After lipofection cells were cultured 72h, HDR level was measured with flow cytometry of the restored GFP-flourescence in case of successful HDR.

Results: We revealed that *MAD2L2* knockdown increases HDR approximately 5 times (10.9% vs 2.1% control HDR level, $p = 0.027$). However, we observed almost four-fold (4.78% vs 1.3%, $p = 0.0067$) decrease of HDR after *NUDT16L1* overexpression.

Conclusions: Thus, we reached 5-fold enhancement of HDR that can be used in editing of pathogenic human mutations. Decrease of HDR due to *NUDT16L1* overexpression gives new information about the protein, and can be used in further studies of *NUDT16L1*. The study was supported by the grant #19-34-90130 of Russian Foundation for Basic Research (RFBR).

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P15.015.C Targeting clinically significant dark regions of the human genome with high-accuracy, long-read sequencing

Ian McLaughlin, *John Harting, Zev Kronenberg, Cheryl Heiner, Lori Aro*

Pacific Biosciences, Menlo Park, CA, USA.

Introduction: There are many clinically important genes in "dark" regions of the human genome. These regions are characterized as dark due to a paucity of NGS coverage as a result of short-read sequencing or mapping difficulties. Low NGS sequencing yield can arise in these regions due to the presence of various repeat elements or biased base composition while inaccurate mapping can result from segmental duplications. Long-read sequencing coupled with an optimized, robust enrichment method has the potential to illuminate these dark regions.

Materials and Methods: Using PacBio highly accurate long-read (HiFi) sequencing, coupled with a long-PCR targeted enrichment method, we investigated two important dark region genes that are challenging to accurately type with short-read sequencing due to associated pseudogenes: *CYP21A2*, responsible for congenital adrenal hyperplasia, and *GBA*, responsible for Gaucher disease. For each gene, our aim was to cover regions of pathogenic mutations in a single contiguous sequence or set of sequences that can be assayed in a single reaction. Coriell samples containing known pathogenic mutations were studied in replicate. For each target region, SMRTbell libraries were generated from pooled amplicons and sequenced on a PacBio Sequel II System.

Results: All pathogenic *CYP21A2* and *GBA* variants were accurately called in the test samples. These variants included whole-gene deletions, gene duplication, gene fusions, recombinant exons, and phased compound heterozygotes.

Conclusions: We demonstrate that HiFi sequencing can enable accurate characterization of clinically important dark regions of the human genome that are typically inaccessible to short-read sequencing.

I. McLaughlin: A. Employment (full or part-time); Significant; Pacific Biosciences. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Pacific Biosciences. **J. Harting:** A. Employment (full or part-time); Significant; Pacific Biosciences. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Pacific Biosciences. **Z. Kronenberg:** A. Employment (full or part-time); Significant; Pacific Biosciences. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Pacific Biosciences. **C. Heiner:** A. Employment (full or part-time); Significant; Pacific Biosciences. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Pacific Biosciences. **L. Aro:** A. Employment (full or part-time); Significant; Pacific Biosciences. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Pacific Biosciences.

P15.016.D Microfluidics for micro clinical samples: Lowering the limits of PCR-free WGS sample sizes through automation

*Kate Cunningham[1], Beatriz Rodríguez-Alonso[2,3,4], **Adam Barner**[1], Eugenia Carvajal[1], Nathan Hoverter[5], Ting Wang[1], Kaitlin Chaung[6], Shweta Belur[2,3,4], Mais Jebrail[1], Severine Margeridon[1], Greg Gonye[7], Eric Chow[6], Aleksandar Rajkovic[2,3,4], Foteini Christodoulou[1]*

[1]Miroculus Inc, San Francisco, CA, USA, [2]Department of Pathology, University of California San Francisco, San Francisco, CA, USA, [3]Department of Obstetrics, Gynecology and Reproductive Sciences, University of California San Francisco, San Francisco, CA, USA, [4]Institute of Human Genetics, University of California San Francisco, San Francisco, CA, USA, [5]Purigen Biosystems, Inc, Pleasanton, CA, USA, [6]UCSF, Center for Advanced Technology (CAT), San Francisco, CA, USA, [7]Purigen Biosystems, Inc., Pleasanton, CA, USA.

The promise of personalized medicine is best captured in the concept of preimplantation genetic testing (PGT). Current methodologies fall short due to inability to fully sequence embryos to detect all pathogenic variants. To maximize healthy baby outcomes PCR-free Whoe Genome Sequencing (WGS)is desired, but nearly impossible due to the limited amount and quality of samples. To address these challenges, two novel microfluidic technologies were integrated for efficient DNA extraction and PCR-free WGS library production.

Using isotachophoresis-based DNA extraction (Ionic® Purification System, Purigen Biosystems) gDNA was extracted from down to 2,500 cells followed by microfluidic PCR-free library preparation (Miro CanvasTM, MicroculusTM). WGS (20X coverage) from GM12878 cells revealed comparable results for the automated vs manual methods (≥Q30 87.8% vs 87.9%, alignment 99.7% vs 99.7%). The automated workflow detected ~90% of the same SNVs as the manual methods. Well-characterized variants were identifiable: 1) G-to-A transition in CYP2C19 exon5 in GM12878, 2) F508 deletion mutation in CTFR in GM07339, 3) R553X mutation in GM07461. Miro Canvas libraries present superior sequencing metrics than manually prepped libraries when PCR-free assay receives very low gDNA input amounts.

This novel combination of technologies allows for hands-off, PCR-free library preparation for WGS of low cell number samples. We have successfully detected known pathogenic variants across different cell lines using as few as 2,500 cells, providing a path forward to achieving the full potential of WGS inclinical scenarios where samples are small, rare, and irreplaceable.

K. Cunningham: A. Employment (full or part-time); Significant; Miroculus, Inc.. **B. Rodríguez-Alonso:** None. **A. Barner:** None. **E. Carvajal:** A. Employment (full or part-time); Significant; Miroculus, Inc. **N. Hoverter:** A. Employment (full or part-time); Significant; Purigen Inc. **T. Wang:** A. Employment (full or part-time); Significant; Miroculus, Inc.. **K. Chaung:** None. **S. Belur:** None. **M. Jebrail:** A. Employment (full or part-time); Significant; Miroculus, Inc. **S. Margeridon:** A. Employment (full or part-time); Significant; Miroculus, Inc. **G. Gonye:** A. Employment (full or part-time); Significant; Purigen Biosystems, Inc.. **E. Chow:** None. **A. Rajkovic:** None. **F. Christodoulou:** A. Employment (full or part-time); Significant; Miroculus, Inc..

P15.017.A Impact of clean-up kits on DNA sequencing quality and efficiency

Tracy Andrews

Beckman Coulter United Kingdom Limited, High Wycombe, United Kingdom.

The applications of DNA sequencing in biological research are growing. A significant driver of this growth is Next-Generation Sequencing (NGS), a modern DNA sequencing technology instrumental in achieving complete DNA sequences or genomes of humans, many animals, plants, and microbial species. Clean-up kits are a pivotal part of the NGS process. They have an immediate impact on efficiency and on quality, as well as key impacts downstream. Clearly, it is not worth jeopardizing entire DNA sequencing operations and more, simply to save pennies on the price per sample of cheaper but lower performance, poorer quality, and less efficient kits. It is therefore critical to examine a DNA sequencing clean-up kit before selection and compare its

overall performance with others, either when starting NGS operations or when considering changing to a different clean-up kit solution. While clean-up kit cost is always a factor, switching to cheaper or other products is not necessarily more advantageous and focusing exclusively on price can have highly undesirable consequences. Here we describe 5 factors and their possible impacts to evaluate before switching a clean-up chemistry.

T. Andrews: A. Employment (full or part-time); Significant; Beckman Coulter United Kingdom Limited.

P15.018.B Genetic carrier screening for monogenic disorders prevalent among yakut ethnic group using population specific low density DNA microarray

Mira Savvina, *Nadezhda Maksimova, Aitalina Sukhomyasova*

North-Eastern federal university, Yakutsk, Russian Federation.

Genetic studies of population isolates have great potential to provide a unique insight into genetic differentiation and phenotypic expressions. Yakut population represents genetic isolates with its unique geographic situation and specific history that have resulted in a relatively higher prevalence of genetic disorders that caused by specific mutation rarely found or cannot be found in other populations. The heterozygous carrier frequency of mutations 4582_4583insT in *CUL7* gene, c.5741G>A in *NBAS* gene, c.806C>T in *DIA1* gene, c.1090G>C in *FAH* gene, c.-23+1G>A in *GBJ2* gene causing five monogenic disorders prevalent among yakut ethnic group: 3-M syndrome, SOPH-syndrome, Tyrosinemia type 1, Methaemoglobinaemia type 1, Nonsyndromic hearing loss and deafness (DFNB1) type 1A respectively was estimated using low density DNA microarray. After genotyping of 120 samples from healthy individuals of yakut origin the frequency of heterozygous carriage were estimated: mutation 4582_4583insT in *CUL7* gene -2%, mutation c.5741G>A in NBAS gene-1,6%, c.806C>T in *DIA1* gene-*2,5%*, c.-23+1G>A in *GBJ2* gene -2,9%. The data was validated by real-time PCR and PCR-RLFP methods. The developed population specific low density oligonucleotide microarray can be considered as an alternative low-cost tool for heterozygous carrier screening and genetic diagnostics in republic of Sakha (Yakutia). The research is conducted under the state target program: project FSRG-2020-0014 "Genomic of Arctic: epidemiology, hereditary and pathology"

M. Savvina: None. **N. Maksimova:** None. **A. Sukhomyasova:** None.

P15.020.D Extracellular vesicles with specific surface proteins are associated with decreased body fat and obesity

Ranran Zhai[1], *Xia Shen*[1,2], *Lu Pan*[3], *Zhijian Yang*[1], *Ting Li*[1], *Zheng Ning*[1,3], *Yudi Pawitan*[3], *James F. Wilson*[2], *Di Wu*[4]

[1]*Sun Yat-sen University, Guangzhou, China,* [2]*University of Edinburgh, Edinburgh, United Kingdom,* [3]*Karolinska Institutet, Stockholm, Sweden,* [4]*Vesicode AB, Stockholm, Sweden.*

Obesity has a highly complex genetic architecture, making it difficult to understand the underlying disease mechanisms, despite the large number of loci discovered via genome-wide association studies (GWAS). Omics technologies provide new perspectives to better understand disease processes. As a proxy of cellular biology, extracellular vesicles (EVs) are useful for studying cellular regulation of complex phenotypes. Here, in a cohort of 96 individuals from Orkney, we utilized a novel technology to detect 113 surface proteins across millions of individual EVs in each individual's plasma sample and conducted GWAS of the abundance of EVs with different protein-protein combinations. Integrating the results with existing obesity GWAS, we inferred 66 types of EVs carrying combinations of 12 surface proteins to be associated with adiposity-related traits such as waist circumference. We verified such associations between the abundance of particular EVs and body fat measured by DEXA scans. GWAS of EVs carrying two surface protein markers revealed genome-wide significant cis-EV-QTL, even given the relatively tiny sample size. Our findings provide evidence that EVs with specific surface proteins are genetically and phenotypically associated with obesity, guiding future EV biomarker discovery.

R. Zhai: None. **X. Shen:** None. **L. Pan:** None. **Z. Yang:** None. **T. Li:** None. **Z. Ning:** None. **Y. Pawitan:** None. **J.F. Wilson:** None. **D. Wu:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; Vesicode AB.

P15.021.A Improving the efficiency of *EGFP* editing by CRISPR-Cas9 ribonucleoprotein complexes

Yana Slesarenko, *Alessya Bykonya, Arina Anuchina, Milyausha Zaynitdinova, Alexander Lavrov, Svetlana Smirnikhina*

Research Centre for Medical Genetics, MOSCOW, Russian Federation.

Introduction: The issue of optimal delivery of CRISPR-Cas9 into cells remains unresolved. Fortunately, the delivery efficiency could be increased using sgRNA and Cas9 protein in form of the ribonucleoprotein (RNP) complex. In this study, we compared the efficiency of indels formation in the *EGFP* gene and the c.337delG mutation correction efficiency in this gene via delivery of CRISPR-Cas9 in the plasmids and RNP.

Materials and methods: We used HEK293T cell line, *EGFP* plasmid without mutation (introduction of indels) and with the c.337delG mutation (used ssODN to correct the mutation). RNP complexes consisted of SpCas9 protein (NEB) and sgRNA to *EGFPwt* or *EGFPmut* (Guide-it sgRNA IVT Kit, Takara Bio). RNP lipofection was performed with Lipofectamine CRISPRMAX and with Lipofectamine 2000 for plasmids and ssODN. The efficiency of indel formation and mutation correction was analysed by flow cytometry. The Mann-Whitney test and t-test were used to find the significance of differences in editing efficiency.

Results: The results showed that the efficiency of indels formation in the *EGFP* using RNP increased by 2.8 fold ($p < 0.05$). The average efficiency of mutation correction was 27.81% using RNP and 4.85% with plasmid delivery ($p < 0.05$). Further optimization of the delivery protocols for plasmids and RNP increased the efficiency of mutation correction in *EGFP* to 16.99% ($p < 0.05$) and 35.22%, respectively.

Conclusion: The results of the work indicate the efficiency of indels formation in the *EGFP* gene and correcting the c.337delG mutation in this gene significantly increased using RNP for CRISPR-Cas9 delivery.

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P15.022.B Large gene panel sequencing in clinical setting - experience from 3044 patients

Hanno Roomere[1], *Ülle Murumets*[1], *Sander Pajusalu*[1,2], *Ustina Šamarina*[1], *Tiina Kahre*[1,2]

[1]*Department of Clinical Genetics, United Laboratories, Tartu University Hospital, Tartu, Estonia,* [2]*Department of Clinical Genetics, Institute of Clinical Medicine, Tartu University, Tartu, Estonia.*

Introduction: The need for genetic testing has grown in recent years. Illumina's TruSight One Expanded (TSO) panel targeting ~6700 genes has been used to diagnose patients with suspected monogenic disorders in Estonia. Physicians and laboratories can choose to analyse all of the genes on the panel or focus on a specific genes or subpanels only.

Materials and Methods: Altogether 3044 individuals were analysed using TSO panel during 2018-2020. The patients were referred to testing by many doctors from different clinics and specialities.

Results: Molecular variant was reported for 699 (23%) individuals. (Likely) pathogenic variants were found in 505 (17%) of cases and variants of unknown significance (VUS) in 194 (6%). VUS were reported only when further studies to confirm the pathogenicity were possible. The most common indications for testing were mental retardation, 862 cases (14% positive); metabolic disorders, 590 (31%); epilepsy, 400 (22%); muscle diseases, 237 (35%); mitochondrial diseases, 236 (25%). Surprisingly, there were no findings from Parkinson's disease and dystonia gene panel. Patients were consented for reporting secondary findings. In 18 patients out of 1255, (1.4%) a pathogenic variant was found in ACMG 59 gene list. In addition, physicians order a reanalysis for 126 previously negative cases, which lead to 13 (10.3%) additional molecular diagnoses.

Conclusions: TSO panel is a powerful tool for detection of rare genetic disorders in clinical practice, with about 25% of diagnostic sensitivity. An accurate clinical diagnosis and provision of relevant clinical information increases the diagnostic yield.

H. Roomere: None. **Ü. Murumets:** None. **S. Pajusalu:** None. **U. Šamarina:** None. **T. Kahre:** None.

P15.023.C *GRIN2B* novel de-novo variants the cause of patients with generalized severe hypotonia as primary referral condition

*FAIDON NIKOLAOS TILEMIS[1,2], **NIKOLAOS M. MARINAKIS**[1], MARIA SVINGOU[1], KYRIAKI KEKOU[1], KONSTANTINA KOSMA[1], MARIA ROSER PONS[3], JOANNE TRAEGER-SYNODINOS[1]*

[1]Laboratory of Medical Genetics, St. Sophia's Children's Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece, [2]Research University Institute for the Study and Prevention of Genetic and Malignant Disease of Childhood, St. Sophia's Children's Hospital, National and Kapodistrian University of Athens, Athens, Greece, [3]1st Department of Pediatrics, St. Sophia's Children's Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece.

Introduction: The *GRIN2B* gene plays a crucial role in normal neuronal and brain development. *GRIN2B* is member of the *N-methyl*-D-aspartate receptor (NMDAR) gene family and is implicated in many cases of neurological disorders. The most common *GRIN2B*-related disorders, which are inherited in an autosomal dominant manner, include mild to profound developmental delay/intellectual disability and muscle tone abnormalities, such as hypotonia.

Materials and Methods: Three patients with prominent central hypotonia, were referred to the Laboratory of Medical Genetics, following clinical evaluation, pre-test counselling and signed consent. Previous testing for Spinal Muscular Atrophy (SMA), Prader-Willi and karyotype were negative. Whole Exome Sequencing (WES, ~19,000 genes) was implemented using Human Core Exome kit (Twist Bioscience) for library preparation and a NextSeq-500 system (Illumina) for sequencing. Bioinformatic analysis was performed using BWA and GATK algorithms, the VarAFT annotation and filter tool and ExomeDepth for CNV detection. Variants were evaluated using VarSome database and categorised according to ACMG guidelines.

Results: Two novel *de-novo* heterozygous nucleotide variants c.1606G>A and c.2459G>C and one *de-novo* heterozygous large deletion, encompassing the entire *GRIN2B* gene, were identified through WES. All variants were classified as Pathogenic according to ACMG guidelines.

Discussion: These findings support that *GRIN2B* variants are frequently and strongly associated with prominent hypotonia of central origin. Also, this study indicates the importance of calling CNVs from exome sequence data using CNV calling algorithms, such as ExomeDepth, to increase the diagnostic yield of WES. The combined detection of point mutations and CNVs makes WES a very useful diagnostic test for patients with hypotonia.

F.N. Tilemis: None. **N.M. Marinakis:** None. **M. Svingou:** None. **K. Kekou:** None. **K. Kosma:** None. **M.R. Pons:** None. **J. Traeger-synodinos:** None.

P15.024.D High resolution HLA typing with NGSgo MX6_1 and PacBio HiFi sequencing

*Ian McLaughlin[1], John Harting[1], Sake van Wageningen[2], **Hanneke Merkens**[2], Loes A. L. van de Pasch[2], Maarten Penning[2], Erik H. Rozemuller[2]*

[1]Pacific Biosciences, Menlo Park, CA, USA, [2]GenDx, Utrecht, Netherlands.

Reliable NGS-based HLA typing requires high-quality reads of sufficient length to resolve ambiguities. Short read sequencing has the advantage of being of high quality but can sometimes lead to ambiguous HLA typing due to limited phasing. This study investigates whether PacBio HiFi sequencing on a Sequel II System (Pacific Biosciences) allows for generating long, high quality reads with full phasing, supporting reliable and unambiguous HLA typing.

A 58 gDNA sample panel was amplified using the NGSgo®-MX6-1 amplification strategy for HLA-A, B, C, DRB1, DQB1 and DPB1 (GenDx). Products were processed in a PacBio library preparation workflow using SMRTbell Express Template Prep Kit 2.0 and barcoded overhang adapters. Libraries were sequenced on a Sequel II System, and data was analyzed in NGSengine®.High quality data was generated for all samples. As promised by long-read sequencing, all loci could be completed phased which resolves genotype ambiguities reported by short read sequencing, even for samples with sparsely distributed heterozygous positions as identified in DPB1. The typing results of all loci in all samples were 100% concordant to the pre-type information. Depth of coverage was constant across the complete amplicon.

PacBio HiFi sequencing of amplicons generated with NGSgo-MX6-1 results in high-quality, long read sequences of HLA. The long reads contribute to a constant depth of coverage combined with full phasing and low noise levels, allowing for reliable HLA typing with limited ambiguities. This new long read sequencing strategy is an attractive alternative to current short read sequence technologies with limited phasing capacity.

I. McLaughlin: A. Employment (full or part-time); Significant; Pacific Biosciences. **J. Harting:** A. Employment (full or part-time); Significant; Pacific Biosciences. **S. van Wageningen:** A. Employment (full or part-time); Significant; GenDx. **H. Merkens:** A. Employment (full or part-time); Significant; GenDx. **L.A.L. van de**

Pasch: A. Employment (full or part-time); Significant; GenDx. **M. Penning:** A. Employment (full or part-time); Significant; GenDx. **E. H. Rozemuller:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; GenDx.

P15.025.A Estimating the X chromosome-mediated risk for developing Alzheimer's disease

Carmel Armon, *Sharon Wolfson, Rivka Margalit, Liraz Avraham, Yael Bugen, Amir Cohen, Adi Meiri, Ran Shorer*

Tel Aviv University School of Medicine and Shamir (Assaf Harofeh) Medical Center, Zeriffin, Israel.

Background: Parental lineage has been shown to increase the risk of Alzheimer's disease (AD) in the offspring, with greater risk attributed to maternal lineage. While 40 genes/loci have been linked to the risk of developing AD, none has been found on the X chromosome.

Methods: We reviewed retrospectively records of patients aged 55-80 years presenting to our memory disorders clinic with amnestic mild cognitive impairment (aMCI) or early AD between May 2015-September 2020. We estimated the risk for developing AD mediated by the X chromosome in a subgroup of late-onset patients with aMCI or early AD and unilateral ancestral history of AD or dementia by analyzing their numbers (a-d) defined as follows:

Probands	Paternal side affected	Maternal side affected
Women	a	b
Men	c	d

The odds ratio OR = (a:b)/(c:d) estimates the relative risk conferred by the X chromosome, controlling for confounders. The estimated proportion of risk mediated by the X chromosome is calculated as (OR-1)/)OR.

Results: 40 women aged 66.1± 5.1 years (mean±standard deviation) and 31 men aged 68.1±6.5 were identified. The OR was (18:22)/(6:25) = 3.4, (95% confidence interval 1.1-10.1; p = 0.027). The estimated proportion of genetic risk borne by the X chromosome in this population is 70%.

Conclusions: Our numbers are small and the findings preliminary, requiring replication. This approach may provide an estimate for the proportion of risk mediated by the X chromosome in individuals who develop AD with unilateral ancestral lineage, and is generalizable.

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P15.026.B Using next generation sequencing and liquid biopsy techniques to provide a new option for a patient with colorectal cancer to monitor cancer recurrence after surgery

Chia Cheng Hung[1], *Tze Kang Lin*[1,2], *Yu Lin Lin*[3], *Chieh Wei Huang*[4], *Chang Wei Yeh*[4], *Hwei Ming Wang*[5], *Yi Ning Su*[1,6]

[1]*Sofiva Genomics, Co., Ltd., Taipei, Taiwan,* [2]*Graduate Institute of Clinical Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan,* [3]*Division of Hematology and Oncology, Department of Internal Medicine, Cardinal Tien Hospital, Taipei, Taiwan,* [4]*National Center for High Performance Computing, National Applied Research Laboratories, Hsinchu, Taiwan,* [5]*Division of Colorectal Surgery, China Medical University Hospital, Taichung, Taiwan,* [6]*Dianthus Maternal Fetal Medicine Clinic, Taipei, Taiwan.*

Background: Liquid biopsy is a new technology to analyze circulating tumor DNA (ctDNA) from tumors. Frequency detecting of driver mutations in ctDNA should be an alternative option for monitoring disease progression.

Method: The patient was diagnosed with colorectal cancer in December 2017 by iFOBT. We used next-generation sequencing (NGS) based 197 targeted genes panel assay and sequenced on an Illumina NextSeq 550.

Results: The tumor markers, CEA19-9 and CEA, were tested and the results were 5.11 ng/ml and 4.23 U/ml, respectively. In February 2018, the result of tissue biopsy detected two mutations, APC p.Gln1406* (55.2%) and TP53 p.Asn239Asp (58.0%). The liquid biopsy also detected the same mutations with mutation rates 0.44% and 0.38%, respectively. The case underwent surgical treatment and the postoperative report showed T3N0M0 in March 2018. After received adjuvant postoperative treatment with 5-FU in April 2018, intensive follow-up for CEA19-9 and CEA were 6.18 ng/ml and 2.18 U/ml, respectively. However, the liquid biopspy showed no abnormalities. After a course of treatment, the patients achieved liquid biopsy and medical imaging examine for an average of 2 to 6 months to monitor cancer recurrence. Except for the detection of a new gene mutation (TP53 p.Lys132Glu, 0.19%) in August 2018, the monitoring results of liquid biopsy are all normal in October 2018, April 2019, November 2019, and April 2020. Similarly, CT scan and colonoscopy tracking also found no abnormalities.

Conclusion: In addition to the tumor markers, the liquid biopsy can be an option to continued monitor cancer progression to assist medical imaging examine results.

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P15.027.C CRISPs regulates efficient flagellar energetics and optimal sperm function

Avinash Gaikwad[1,2], *Ashwin Nandagiri*[3], *David Potter*[4], *Ranganathan Prabhakar*[5], *Sameer Jadhav*[6], *Julio Soria*[7], *Reza Nosrati*[5], *Moira O'Bryan*[8]

[1]*Institute of Reproductive Genetics, University of Münster, Münster, Germany,* [2]*School of Biological Sciences, Monash University, Melbourne, Australia,* [3]*IITB-Monash Research Academy, Mumbai, India,* [4]*Monash Micro Imaging - Advanced Optical Microscopy, Monash University, Melbourne, Australia,* [5]*Department of Mechanical & Aerospace Engineering, Monash University, Melbourne, Australia,* [6]*Department of Chemical Engineering, Indian Institute of Technology-Bombay, Mumbai, India,* [7]*Laboratory for Turbulence Research in Aerospace and Combustion (LTRAC), Department of Mechanical & Aerospace Engineering, Monash University, Melbourne, Australia,* [8]*School of BioSciences, University of Melbourne, Melbourne, Australia.*

Introduction: Cysteine-Rlch Secretory Proteins (CRISPs) are highly expressed in the epididymis and are hypothesized to be involved in establishing optimal functional competence. Previously, it has been shown that CRISPs can regulate ion flow via various sperm ion channels, and thus potentially regulate sperm function, including motility.

Materials and methods: We use image-analysis and hydrodynamic calculations to show that epididymal CRISPs significantly influences sperm flagellar beating. This was achieved by

developing an image-analysis algorithm that utilizes proper orthogonal decomposition to study the complex dynamics and beating pattern of the sperm flagella.

Results: Sperm from wildtype mice exhibited rhythmic and sinusoidal flagella beating. By contrast, sperm from *Crisp1*$^{-/-}$ and *Crisp4*$^{-/-}$ knockout mice displayed asymmetric flagellar beating. The changes observed between genotypes were characterized by reconstructing an average beat cycle, which revealed that *Crisp1*$^{-/-}$ sperm possessed an inflexible mid-piece and a highly asymmetric waveform at the distal regions of the flagella. By contrast, the *Crisp4*$^{-/-}$ sperm were constrained along the entire length of the flagella. We found that *Crisp1*$^{-/-}$ and *Crisp4*$^{-/-}$ sperm had altered periodicity of flagellar oscillations between cycles and lowered flagellar amplitude, reduced oscillating frequency of the flagella and reduced rates of energy dissipation along the flagella. Excitingly, these deficits could be largely rescued by the addition of recombinant CRISPs to sperm.

Conclusions: Collectively, the data reveal that CRISPs play a significant role in establishing efficient sperm flagella waveform and function and thus, fertility. This data also suggests the use of recombinant CRISPs may be of benefit in assisted reproductive technologies.

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P15.028.D CoverageMaster: a clinical grade and user oriented CNV caller

Melivoia Rapti[1], *Emmanuelle Ranza*[2], *Stylianos E. Antonarakis*[3], *Federico A. Santoni*[1]

[1]*Centre Hospitalier Universitaire Vaudois (CHUV), Lausanne, Switzerland,* [2]*Medigenome, Swiss Institute of Genomic Medicine, Geneva, Switzerland,* [3]*University of Geneva Medical Faculty, Geneva, Switzerland.*

Copy number variation (CNV) is the most frequent structural alteration in the human genome. Aberrant number of copies of specific genes or genomic regions are known to cause pathogenic conditions. While advances in next generation sequencing (NGS) have provided a standardized way for accurate coding variant analyses through whole exome sequencing (WES), CNV detection still relies on probe-based methods, that are complementary to NGS approaches in clinical diagnostics. Limited arrayCGH resolution, poor MLPA scalability and additive costs are pushing for WES to become the primary strategy for identifying CNVs. However, WES technical issues made it difficult so far to standardize a procedure for CNV detection, mostly because of the high false positive rates. Here, we introduce CoverageMaster (CM), a CNV calling algorithm based on depth-of-coverage maps from aligned short sequence reads. CNVs are inferred with Hidden Markov probabilistic Models at the nucleotide-level in the Wavelet compressed space, while existing methods utilize fixed length windows or exon averages. This approach allows the user to visually inspect the output to further reduce the false positive rate. Indeed, CM provide the graphical representations of the predicted CNVs for all the genes of interest, and optionally, a wig formatted file compatible with USCS Genome Browser for detailed visualization of the target regions.CM is being tested with research and clinical data as a supportive diagnostic tool and preliminary data and already validated cases suggest the concrete possibility of WES-based CNV callers to replace arrayCGH and MLPA in the near future.

M. Rapti: None. **E. Ranza:** None. **S. Antonarakis:** None. **F. Santoni:** None.

P15.029.A Review of mitochondrial genome analysis in over 7,500 patients using clinical grade mtDNA sequencing

Jonna Tallila, *Marita Isokallio, Inka Saarinen, Miko Valori, Ville Kytola, Pauli Siivonen, Pertteli Salmenpera, Massimiliano Gentile, Johanna Sistonen, Tero-Pekka Alastalo, Juha Koskenvuo*

Blueprint Genetics, Espoo, Finland.

Introduction: Mitochondrial diseases are a heterogeneous group of disorders caused by mitochondrial dysfunction. Mixed populations of both normal and mutant mitochondrial DNA can coexist in a single cell (heteroplasmy). The proportion has important consequences in understanding mitochondrial disease, as the heteroplasmy level contributes to the severity of mitochondrial disorders and a phenotypic manifestation occurs only when a critical threshold level is exceeded.

Methods: We developed a highly sensitive and clinically validated mtDNA assay based on hybridization-based capture of mtDNA and next-generation sequencing (NGS) that is able to detect very low heteroplasmy levels of SNVs, INDELs and deletions. The mean read depth across the mitochondrial genome was 18,224x, and 100% of base pairs were covered at least 1000x. Sensitivity to detect SNVs and INDELs with over 10% heteroplasmy was 100%. For SNVs with 5-10% and <5% heteroplasmy levels the sensitivity was 93.3% and 88.9%, respectively.

Results: A diagnostic (pathogenic/likely pathogenic) mtDNA variant was identified in 116 patients, contributing to a diagnostic yield of 1.5%. Not surprisingly, the most common recurrent variant was m.3243A>G (40 patients with heteroplasmy levels ranging from 5.8 to 70.9%), which underlies maternally inherited diabetes, hearing loss, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS). Single large deletions ranging in size from 4.4 to 7.6kb with heteroplasmy levels of 6.8 to 54.5% were observed in 4 cases.

Conclusions: Mitochondrial disorders may be caused by pathogenic variants of genes encoded by either nDNA or mtDNA, thus combining a high-quality mtDNA analysis with routine panel-based diagnostics improves the diagnostic yield.

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P15.030.B Molecular karyotyping with Nanopore sequencing

Pamela Magini[1], *Alessandra Mingrino*[2], *Barbara Gega*[2], *Davide Bolognini*[2], *Gianluca Mattei*[3], *Roberto Semeraro*[2], *Patrizia Mongelli*[1], *Laura Desiderio*[1], *Maria C. Pittalis*[1], *Tommaso Pippucci*[1], *Alberto Magi*[3]

[1]*U.O. Genetica Medica, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy,* [2]*Dipartimento di Medicina Sperimentale e Clinica, Università degli Studi di Firenze, Firenze, Italy,* [3]*Dipartimento di Ingegneria dell'Informazione, Università degli Studi di Firenze, Firenze, Italy.*

Introduction: Copy number variants (CNVs) play important roles in the pathogenesis of several genetic syndromes. Traditional and molecular karyotyping are considered the first-tier diagnostic tests

to detect macroscopic and cryptic deletions/duplications. However, their time-consuming and laborious experimental protocols protract diagnostic times by three to fifteen days. Long read sequencing approaches, such as Oxford Nanopore sequencing (ONS), have the ability to reduce time to results for the detection of CNVs with the same resolution of current state-of-the-art diagnostic tests. We compared ONS to molecular karyotyping for the detection of pathogenic CNVs to demonstrate its clinical utility.

Material and Methods: Genomic DNA was extracted from peripheral blood samples of 7 patients with previously diagnosed causative CNVs of different sizes and allelic fractions. Larger chromosomal anomalies included trisomy 21 and mosaic tetrasomy 12p. Among smaller CNVs we tested two reciprocal genomic imbalances in 7q11.23 (1.367 Mb), a 170 kb deletion encompassing *NRXN1* and mosaic 6q27 (1.231 Mb) and 2q23.1 (408 kb) deletions. DNA libraries were prepared following Oxford Nanopore protocol and sequenced on the GridION device for 48 h. Data were analysed in online mode, using NanoGLADIATOR.

Results: ONS identified all pathogenic CNVs with detection time inversely proportional to size and allelic fraction. Aneuploidies were called after only 30 minutes of sequencing, while 30 hours were needed to call CNVs < 500 kb also in mosaic state (44%).

Conclusions: Through a rapid and simple workflow, Nanopore technology allows the molecular diagnosis of genomic disorders within 30 minutes to 30 hours time-frame.

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P15.031.C Accessing whole human genome methylation through nanopore sequencing: potential for application in human genetics

Catarina Silva[1,2,3], *Miguel Machado*[1], *Sebastião Rodrigues*[2,3], *Luís Vieira*[1,2]

[1]*Technology and Innovation Unit, Department of Human Genetics, National Institute of Health Doutor Ricardo Jorge, Lisbon, Portugal,* [2]*Centre for Toxicogenomics and Human Health (ToxOmics), Genetics, Oncology and Human Toxicology, Nova Medical School, University Nova de Lisboa, Lisbon, Portugal,* [3]*Nova Medical School|FCM, University Nova de Lisboa, Lisbon, Portugal.*

Introduction: Several genetic diseases are associated with cytosine methylation (5′-mC) of CpG dinucleotides. Assessment of whole genome 5′-mC status frequently requires treatment with sodium bisulfite or digestion with methylation-sensitive restriction enzymes. However, modification of DNA sequences can lead to a sub-optimal methylation profile due to technical biases. Here we assessed the potential of nanopore sequencing (NS) to characterize whole genome 5′-mC using native DNA.

Materials and Methods: Genomic DNA extracted from HEL cell line was sequenced in a MinION following a rapid library preparation protocol (Oxford Nanopore Technologies). Methylation calling was performed using the nanopype pipeline. Several R scripts were used to compare NS data with publicly available digital restriction enzyme analysis of methylation (DREAM) and methylation array data.

Results: NS mimicked the expected genomic distribution of CpGs at ~10X average genome coverage. A higher number of methylated CpGs was found in shores and shelves compared to CpG islands, compatible with the role of those genomic regions in gene regulation. The correlation of methylation frequencies (MF) was higher with microarray than with DREAM data, and increased to a maximum of ~0.89 at 17X. In contrast to NS, DREAM and microarray data showed a tendency towards extreme and intermediate MF values for CpG shores, shelves and open sea regions.

Conclusions: NS provides unbiased whole genome methylation calling and a faithful representation of the CpG profiles in various genomic contexts. We conclude that NS can be used to study abnormal DNA methylation patterns in genetic diseases (supported by ToxOmics and GenomePT).

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P15.032.D Introduction of a walk-away automated Roche NGS workflow solution: Integrated KAPA Library Preparation, KAPA Target Enrichment and the AVENIO Edge instrument

Persis Wadia, *Joshua Lefkowitz, Edwin Malfarta, Arrezo Moghaddasi, Smriti Sharma, Benjamin Morck, Patrick Ton, Tim Williams, Michael Pintor, Jennifer Dasgupta, Arash Fararooni, Arafat Al-Ariemy, Bill LaRochelle, Pierre-Luc Janvier, Neda Razavi*

Roche, Pleasanton, CA, USA.

Introduction: By automating and simplifying NGS workflow steps, a variety of diagnostic applications become practical and robust, thus increasing the efficiency of precision medicine. The AVENIO Edge instrument provides a complete walk-away automated NGS library prep solution with on-deck QC from extracted nucleic acid with minimal hands-on time and flexibility for KAPA Target Enrichment. Feasibility of an NGS workflow with zero pipetting steps using Roche KAPA Library Preparation and KAPA Target Enrichment reagents with an AVENIO Edge instrument was explored.

Methods: Performance, carry-over contamination rate, turnaround time, DNA quantitation module testing, were representative experiments comparing automated vs. manual capture workflows using Roche KAPA HyperCap Workflow v3.0 reagents on the AVENIO Edge instrument. Representative panels including KAPA HyperExome Panels were tested with pre-capture and post-capture pooling workflows using genomic DNA. Sequencing data was analyzed through internal pipelines and included percent reads on-target, fold-80 base penalty, mean target coverage, total duplicate rate, 90th/10th percentile ratio, mean insert size.

Results: The AVENIO Edge Quant kit results and sequencing metrics were similar between automated and manually prepared samples for all representative panels. No significant differences were observed from reagent lot-to-lot, run to run & between instruments with minimal contamination across runs. Average turn-around-time for 24 sample processing was ~31 hours.

Conclusions: We successfully demonstrated the integration and performance of the complete Roche KAPA HyperCap Workflow v3.0 leveraging KAPA Library Preparation and KAPA Target Enrichment reagents on the AVENIO Edge instrument, enabling the broader adoption of NGS in precision medicine and ultimately improving patient outcomes.

P. Wadia: A. Employment (full or part-time); Significant; Roche. **J. Lefkowitz:** A. Employment (full or part-time); Significant; Roche. **E. Malfarta:** A. Employment (full or part-time); Significant; Roche. **A. Moghaddasi:** A. Employment (full or part-time); Significant; Roche. **S. Sharma:** A. Employment (full or part-time); Significant; Roche. **B. Morck:** A. Employment (full or part-time); Significant; Roche. **P. Ton:** A. Employment (full or part-time); Significant; Roche. **T. Williams:** A. Employment (full or part-time); Significant; Roche. **M. Pintor:** A. Employment (full or part-time); Significant; Roche. **J.**

Dasgupta: A. Employment (full or part-time); Significant; Roche. **A. Fararooni:** A. Employment (full or part-time); Significant; Roche. **A. Al-Ariemy:** A. Employment (full or part-time); Significant; Roche. **B. LaRochelle:** A. Employment (full or part-time); Significant; Roche. **P. Janvier:** A. Employment (full or part-time); Significant; Roche. **N. Razavi:** A. Employment (full or part-time); Significant; Roche.

P15.033.A Monitoring circulating tumor DNA predicts cancer recurrence by using liquid biopsy in a colorectal cancer patient

*Chia Cheng Hung[1], Hwei Ming Huang[2], Tze Kang Lin[1,3], Chieh Wei Huang[4], Chang Wei Yeh[4], **Yi Ning Su**[1,5]*

[1]Sofiva Genomics, Co., Ltd., Taipei, Taiwan, [2]Division of Colorectal Surgery, China Medical University Hospital, Taichung, Taiwan, [3]Graduate Institute of Clinical Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan, [4]National Center for High Performance Computing, National Applied Research Laboratories, Hsinchu, Taiwan, [5]Dianthus Maternal Fetal Medicine Clinic, Taipei, Taiwan.

Background: Liquid biopsy is an alternative tool for discover tumor-specific mutations. The current clinical application is focusing on early prediction of cancer recurrence.

Method: Liquid biopsy via circulating tumor DNA (ctDNA) in blood test results were collected from a 62-year old patient with stage IV (T3N0M1) colorectal cancer after one month, three months and one year of surgery. The targeted sequencing of 197 genes was used for tumor-derived ctDNA analysis by next generation sequencing (NGS).

Results: In September 2019, the patient was found metastatic adenocarcinoma of lung in right upper and right lower lobes. The immunohistochemistry (IHC) staining showed CK7 negative, CK20 positive, CDX-2 positive and TTF-1 negative. Using the mutations detected by liquid biopsy, we found one FBXL7 c.1351 G>A (p. Ala415Thr) mutation and the mutation was 0.89% in October 2019. After three months of surgery (January 2020), we noted the same mutation but the mutation rate was increasing to 0.99%. Another nine mothers later (October 2020), the mutation rate was a roughly 2-fold increase to 1.68%. The percentage of recurrent somatic mutations reveal insight cancer recurrence. The patient was diagnosed as metastatic lung cancer by using PET imaging in November 2020. The liquid biopsy predicts cancer recurrence months earlier than PET imaging.

Conclusion: The usefulness of liquid biopsy for cancer recurrence and metastasis after surgery shows the clinical need to identify patients at high risk. Early detection of mutations using liquid biopsy could be used as a novel and highly sensitive tool for monitoring cancer progression.

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P15.034.B Combining exome/genome sequencing with data repository analysis reveals novel gene-disease associations for a wide range of genetic disorders

***Aida Bertoli-Avella**[1], Krishna Kandaswamy[1], Suliman Khan[1], Natalia Ordonez-Herrera[1], Kornelia Tripolszki[1], Christian Beetz[1], Maria Rocha[1], Alize Urzi[1], Ronja Hotakainen[1], Anika Leubauer[1], Ruslan Al-Ali[1], V. Karageorgou[1], O. Moldovan[2], P. Dias[2], A. Alhashem[3], B. Tabarki[3], M. Albalwi[4,5], A. Alswaid[6], Z. Al-Hassnan[7], N. Al Hashmi[8], L. Al Gazali[9], H. Cheema[10], M. Zaki[11], I. Hüning[12], A. Alfares[4,13], W. Eyaid[14,5], F. Al Mutairi[14,5], M. Alfadhel[14,5], F. Alkuraya[15], N. Al-Sannaa[16], A. AlShamsi[9], N. Ameziane[1], A. Rolfs[1,17], P. Bauer[1]*

[1]CENTOGENE GmbH, Rostock, Germany, [2]Serviço de Genética Médica. Hospital de Santa Maria. Centro Hospitalar Universitário de Lisboa Norte, Lisboa, Portugal, [3]Division of Pediatric Genetics, Department of Pediatrics, Prince Sultan Military Medical City, Riyadh, Saudi Arabia, [4]Pathology and Laboratory Medicine, King Abdulaziz Medical City, Riyadh, Saudi Arabia, [5]King Abdullah International Medical Research Center (KAIMRC), King Saud bin Abdulaziz University for Health Sciences, MNGHA, Riyadh, Saudi Arabia, [6]College of Medicine, King Saud bin Abdulaziz University for Health Sciences. King Abdulaziz Medical City, Riyadh, Saudi Arabia, [7]Department of Medical Genetics, King Faisal Specialist Hospital & Research Center, College of Medicine, Alfaisal University, Riyadh, Saudi Arabia, [8]National Genetic Center, Royal hospital Muscat, Muscat, Oman, [9]Department of Pediatrics, Tawan Hospital, Al-Ain, United Arab Emirates, [10]Pediatric Department of Gastroenterology. Children's Hospital of Lahore Hospital, Lahore, Pakistan, [11]Clinical Genetics Department, Human Genetics and Genome Research Division, National Research Centre, Cairo, Egypt, [12]Institute of Human Genetics. University Hospital Schleswig-Holstein, Lübeck, Germany, [13]Department of Pediatrics, College of Medicine, Qassim University, Riyadh, Saudi Arabia, [14]Division of Genetics, Department of Pediatrics, King Abdullah Specialized Children Hospital, King Abdulaziz Medical City, MNGHA, Riyadh, Saudi Arabia, [15]Department of Genetics, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia, [16]John Hopkins Aramco Health Care, Pediatric Services, Dharan, Saudi Arabia, [17]University of Rostock, Rostock, Germany.

Introduction: Despite comprehensive genetic testing, over half of the patients with genetic diseases remain molecularly undiagnosed. A presumably major reason is that the causative variants are in genes, for which there is insufficient or no evidence for an association with disease. We aim to discover novel gene-disease associations in patients that remained undiagnosed after performing exome/genome sequencing (ES/GS).

Methods: We followed two approaches: i) a patient-centered approach, which after routine diagnostic analysis systematically interrogates variants in genes not yet associated to human diseases, and ii) a gene-variant centered approach. For the latter, we focused on *de novo* variants in patients that presented with neurodevelopmental delay (NDD) and/or intellectual disability (ID), which are the most common reasons for genetic testing referrals. Gene-disease association was assessed using our data repository that combines ES/GS data and Human Phenotype Ontology terms from over 33,000 patients.

Results: We suggest six novel gene-disease associations based on 38 patients with variants in the *BLOC1S1, IPO8, MMP15, PLK1, RAP1GDS1*, and *ZNF699* genes. Furthermore, our results support causality of 31 additional candidate genes. Following the ClinGen guidelines, these 31 gene-disease associations can be upgraded from having "limited" evidence to "moderate" or "strong", based on 56 patients. The phenotypes included syndromic/non-syndromic NDD/ID, oral-facial-digital syndrome, cardiomyopathies, malformation syndrome, short stature, skeletal dysplasia, and ciliary dyskinesia.

Conclusions: Our results demonstrate the value of data repositories, which combine clinical and genetic data, for discovering and confirming gene-disease associations. Genetic laboratories should be encouraged to pursue such analyses for the benefit of undiagnosed patients.

A. Bertoli-Avella: A. Employment (full or part-time); Significant; Centogene GmbH. **K. Kandaswamy:** A. Employment (full or part-time); Significant; Centogene GmbH. **S. Khan:** A. Employment (full or part-time); Significant; Centogene GmbH. **N. Ordonez-Herrera:**

A. Employment (full or part-time); Significant; Centogene GmbH. **K. Tripolszki:** A. Employment (full or part-time); Significant; Centogene GmbH. **C. Beetz:** A. Employment (full or part-time); Significant; Centogene GmbH. **M. Rocha:** A. Employment (full or part-time); Significant; Centogene GmbH. **A. Urzi:** A. Employment (full or part-time); Significant; Centogene GmbH. **R. Hotakainen:** None. **A. Leubauer:** None. **R. Al-Ali:** A. Employment (full or part-time); Significant; Centogene GmbH. **V. Karageorgou:** A. Employment (full or part-time); Significant; Centogene GmbH. **O. Moldovan:** None. **P. Dias:** None. **A. Alhashem:** None. **B. Tabarki:** None. **M. Albalwi:** None. **A. Alswaid:** None. **Z. Al-Hassnan:** None. **N. Al Hashmi:** None. **L. Al Gazali:** None. **H. Cheema:** None. **M. Zaki:** None. **I. Hüning:** None. **A. Alfares:** None. **W. Eyaid:** None. **F. Al Mutairi:** None. **M. Alfadhel:** None. **F. Alkuraya:** None. **N. Al-Sannaa:** None. **A. AlShamsi:** None. **N. Ameziane:** A. Employment (full or part-time); Significant; Centogene GmbH. **A. Rolfs:** A. Employment (full or part-time); Significant; Centogene GmbH. **P. Bauer:** A. Employment (full or part-time); Significant; Centogene GmbH. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Centogene GmbH.

P15.035.C Optical Genome Mapping: where molecular techniques give up

Viola Alesi[1], *Francesca Romana Lepri*[1], *Silvia Genovese*[1], *Valeria Orlando*[1], *Sarah Loddo*[1], *Silvia Di Tommaso*[1], *Matteo Luciani*[2], *Federica Deodato*[3], *Rossella Capolino*[4], *Antonio Novelli*[1]

[1]*Laboratory of Medical Genetics, Translational Cytogenomics Research Unit, Bambino Gesù Children's Hospital, Rome, Italy,* [2]*Haemophilia Centre, Bambino Gesù Paediatric Hospital, Rome, Italy,* [3]*Division of Metabolism, Bambino Gesù Children's Hospital, Rome, Italy,* [4]*Genetics and Rare Diseases Research Division, Bambino Gesù Children's Hospital, Rome, Italy.*

Optical genome mapping (OGM) is a new technology able to provide information about numerical and structural rearrangements at high resolution. We describe three cases in which it allowed an exhaustive molecular diagnosis. The first patient presented with Neurofibromatosis type 1, an autosomic dominant disease caused by variants in *NF1* gene. NGS tested negative, while MLPA analysis detected two non-contiguous deletions involving *NF1* exons 4-7 and 31-36 respectively. OGM detected the two known microdeletions, along with a 56 Kb intragenic inversion, having the two deletions as breakpoints. The second patient had hemophilia A, an X-linked recessive condition caused by mutation in *F8* gene. NGS and MLPA tested negative. OGM analysis showed a 556 Kb inversion in Xq28, whose proximal breakpoint interrupted *F8* gene. The third patient presented with an Alpha-mannosidosis disease, a recessive genetic condition caused by mutations in *MAN2B1*. NGS detected a homozygous variant in *MAN2B1*, segregated only from the mother, while SNP-array tested negative. A 5.3 Kb paternally inherited deletion was detected by OGM, involving the 3' portion of *MAN2B1*. OGM represents a powerful upgrade to cytogenetics providing genome-wide data not achievable with other techniques. In the presented cases, OGM was used to detect cryptic rearrangements, explaining clinical phenotype. The development of molecular techniques and the stasis of cytogenetics have led to underestimate the causative role of structural micro-rearrangements. This new technology will provide a wide overview of genomic complexity and will help both in addressing a diagnosis and in underlining the molecular mechanism of genomic disease.

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P15.036.D Gene editing strategy for alpha-1 antitrypsin deficiency through CRISPR-cas9 in liver organoids

Sara Perez-Luz[1], *Gema Gomez-Mariano*[1], *Ignacio Perez de Castro*[1], *Iago Justo*[2], *Alberto Marcacuzco*[2], *Loreto Hierro*[3], *Cristina Garfia*[3], *Beatriz Martínez-Delgado*[1]

[1]*IIER, Madrid, Spain,* [2]*Hospital 12 Octubre, Madrid, Spain,* [3]*Hospital La Paz, Madrid, Spain.*

Introduction: Alpha-1 Antitrypsin deficiency (AATD) is an inherited condition characterized by reduced levels of serum AAT due to mutations in *SERPINA1* gene. More than 90% of severe deficiency patients are homozygous for PiZ (Glu342Lys) mutation located in exon 5. The PiZ allele causes the AAT protein to polymerize in hepatocytes, limiting secretion into the circulation, resulting in plasma levels 10% to 15% of the levels of normal M homozygotes. Liver organoids are an interesting model to develop a gene edition CRISPR/Cas9 strategy as a gene therapy to treat AATD.

Materials and Methods: Using HITI technology (Homology-Independent Targeted Integration) we have design a single-stranded RNA guide (gsRNA) to target cas9 endonuclease to intron 4-5 and introduced a wild-type exon 5 into the *SERPINA1* gene of PiZ organoids. CRISPR/Cas9 machinery was delivered into the organoids using the NEPA21 electroporator.

Results: We have first tested the CRISPR/Cas9 machinery in the hepatocarcinoma cell line HepG2 to optimize conditions. In organoids, we have produced several pools in which CRISPR/Cas9 activity (insertions and deletions, Indels) was measured by TIDE analyses, being one single nucleotide deletion the most frequent event when using this sgRNA guide. We are now testing whether minicircles containing the correct exon 5 are capable of restoring alpha-1 antitrypsin levels in the genomic-edited organoids.

Conclusions: These gene-edited organoids could be an ideal model for the development of a gene therapy for DAAT.

S. Perez-Luz: None. **G. Gomez-Mariano:** None. **I. Perez de Castro:** None. **I. Justo:** None. **A. Marcacuzco:** None. **L. Hierro:** None. **C. Garfia:** None. **B. Martínez-Delgado:** None.

P15.037.A Personal Automation for Whole Genome Sequencing: Evaluating Digital Microfluidics Across Two Different PCR-free Protocols

Eugenia Carvajal[1], *Adam Barner*[1], *Julia Yoo*[1], *Preetham Hosur*[1], *Michely Ternadi*[1], *Tom Howd*[2], *Tim Desmet*[2], *Fay Christodoulou*[1], *Mais Jebrail*[1], *Severine Margeridon*[1]

[1]*Miroculus Inc, San Francisco, CA, USA,* [2]*Broad Institute Genomics Platform, Cambridge, MA, USA.*

There is increasing demand for PCR-free WGS (e.g. rapid diagnosis of newborns, Tumor/Normal somatic sequencing) enabling identification of more variants without amplification-associated artifacts. Traditional automation requires commitment to a small set of protocols, necessitating high throughput and batching, to justify the investment of time, space, and capital.

Fully-automated library construction on Miro CanvasTM from MiroculusTM uses electromechanical forces for dispensing, moving reagents, mixing, thermocycling, magnetic bead clean-ups, and eluting on a single-use electronics-free cartridge.

PCR-free WGS protocols were compared to manual and high-throughput liquid handler library preparation: 1) Miro PCR-free

WGS Library Prep Kit with mechanical fragmentation, 2) Illumina DNA PCR-free Prep Kit with tagmentation.

Miro PCR-free WGS libraries (n = 178) were prepared using sheared gDNA inputs (75-500ng) from multiple sources (NA12878 and donor blood samples). Twenty-six Illumina DNA PCR-free libraries were generated using NA12878 inputs (50-500ng).

Miro Canvas libraries demonstrated equivalent or better sequencing metrics compared to both manual efforts and a high-throughput plate-based liquid handler using the same kit. Miro PCR-free WGS libraries consistently achieved >95% bases at 20X coverage and >98%HetSNP sensitivity with 109Gb sequenced across inputs (100-500ng). Libraries from the liquid handler system required 5Gb more to achieve the same.

Sequencing Illumina DNA PCR-free libraries demonstrated equivalent insert size, coverage and %excluded total metrics compared to manually-prepared libraries with average insert size of 425bp and average yield of 10nM (300ng-500ng input).

With Miro Canvas, "on-demand" automation for WGS PCR-free protocols using mechanical fragmentation or tagmentation allows for more consistent, higher quality WGS libraries.

E. Carvajal: A. Employment (full or part-time); Significant; Miroculus, Inc. **A. Barner:** A. Employment (full or part-time); Significant; Miroculus, Inc. **J. Yoo:** A. Employment (full or part-time); Significant; Miroculus, Inc. **P. Hosur:** A. Employment (full or part-time); Significant; Miroculus, Inc. **M. Ternadi:** A. Employment (full or part-time); Significant; Miroculus, Inc.. **T. Howd:** None. **T. Desmet:** None. **F. Christodoulou:** A. Employment (full or part-time); Significant; Miroculus, Inc. **M. Jebrail:** A. Employment (full or part-time); Significant; Miroculus, Inc. **S. Margeridon:** A. Employment (full or part-time); Significant; Miroculus, Inc..

P15.038.B Determining if polygenic scores provide clinical utility

Sowmiya Moorthie[1,2], *Alison Hall*[1], *Joanna Janus*[1], *Tanya Brigden*[1], *Chantal Babb de Villiers*[1], *Laura Blackburn*[1], *Mark Kroese*[1]

[1]*PHG Foundation, Cambridge, United Kingdom,* [2]*Cambridge Public Health, Cambridge, United Kingdom.*

Introduction: Polygenic scores (PGS) have been developed as the mechanism by which knowledge of common variants can be used to investigate genetic contributions to disease risk. Several clinical applications have been discussed. However, demonstrating utility is one of the key barriers to implementation and uptake.

Materials and methods: We applied our expert knowledge of genetic test evaluation, regulation and implementation to PGS analysis to identify potential issues from these perspectives. Our investigation was from the viewpoint of hypothetical implementation of PGS analysis as a novel biomarker test within the UK National Health Service. We also reviewed published and grey literature, and conducted interviews of key individuals using a semi-structured process to understand differing perspectives on the utility of PGS.

Results: Determining the utility of PGS is linked to understanding the nature of the test offered and its intended purpose. This extends beyond demonstration of the performance characteristics of a PGS model and requires consideration of the test, its intended purpose and context of use, in order to examine the implications of test use on healthcare pathways. Our analysis indicates that a lack of clarity on these aspects along with uncertainties in how such tests are regulated are impeding assessment of their utility and therefore their implementation.

Conclusion: We were able to outline factors that impact on considerations of utility of PGS use in healthcare. Clear articulation of proposed applications, specifying the clinical context of use and target population are important in developing evidence towards demonstrating the utility of PGS tests.

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P15.039.C Deep, rapid and unbiased plasma proteomics with Proteograph™ enables proteogenomic studies with differential analysis of proteoforms

Margaret K. R. Donovan, *John E. Blume, Marwin Ko, Ryan W. Benz, Theodore L. Platt, Juan C. Cuevas, Serafim Batzoglou, Asim Siddiqui, Omid C. Farokhzad*

Seer, Inc., Redwood City, CA, USA.

Introduction: Comprehensive coverage of the proteome remains elusive because of proteoforms arising from alternative splicing, allelic variation, and protein modifications. Protein variations are critical to protein function, expanding our knowledge of biological states of diseases, which requires unbiased protein coverage at sufficient scale. Scalable, deep and unbiased proteomics studies have been impractical due to cumbersome and lengthy workflows required for complex samples, like blood plasma. Here, we demonstrate the power of Proteograph in a proof-of-concept proteogenomic analysis of 80 healthy controls and 61 early-stage non-small-cell lung cancer (NSCLC) samples to dissect differences between protein isoforms arising from alternative gene splicing, as well as the identification of novel peptides arising from allelic variation.

Materials, Methods and Results: Processing the 141 plasma samples with Proteograph yielded 21,959 peptides corresponding to 2,499 protein groups. Using peptides with significant abundance differences ($p < 0.05$; Benjamini-Hochberg corrected), we extracted proteins comprised of peptides where at least one peptide had significantly higher plasma abundance, and another significantly lower plasma abundance in controls vs. cancer, resulting in a set of sixteen proteins. For three of these proteins, the abundance variation is putatively explained by underlying protein isoforms. To identify protein variants, we performed exome sequencing on 29 individuals from the NSCLC study, created personalized mass spectrometry search libraries for each individual, and identified 464 protein variants.

Conclusions: Proteograph can generate unbiased and deep plasma proteome profiles that enable identification of protein variants and peptides present in plasma, at a scale sufficient to enable population-scale proteomic studies.

M.K.R. Donovan: A. Employment (full or part-time); Significant; Seer, Inc. **J.E. Blume:** A. Employment (full or part-time); Significant; Seer, Inc. **M. Ko:** A. Employment (full or part-time); Significant; Seer, Inc. **R.W. Benz:** A. Employment (full or part-time); Significant; Seer, Inc. **T.L. Platt:** A. Employment (full or part-time); Significant; Seer, Inc. **J.C. Cuevas:** A. Employment (full or part-time); Significant; Seer, Inc. **S. Batzoglou:** A. Employment (full or part-time); Significant; Seer, Inc. **A. Siddiqui:** A. Employment (full or part-time); Significant; Seer, Inc. **O.C. Farokhzad:** A. Employment (full or part-time); Significant; Seer, Inc..

P15.040.D Rare disease case-finding using a digital tool in UK primary care - a pilot study

William Evans, ***Orlando Buendia****, Connor Toal, Pradeep Ravichandran, Lara Menzies*

Mendelian Ltd, London, United Kingdom.

Introduction: This study implemented MendelScan, a primary care rare disease case finding tool, into a UK NHS population. Rare disease diagnosis is a key priority of the UK Rare Disease Framework.

Methods: A UK primary care locality with 68,705 patients was examined. MendelScan encodes diagnostic/screening criteria for multiple rare diseases, mapping clinical terms to appropriate SNOMED CT codes (UK primary care standardised clinical terminology) to create digital algorithms. These algorithms were applied to a pseudo-anonymised structured data extract of the electronic health records (EHRs) in this locality to "flag" 'at risk patients who may require further evaluation. All flagged patients then underwent internal clinical review; for those that passed, a report was returned to their GPs.

Results: 55 of 76 disease criteria flagged at least one patient.227 (0.33% of the total population) patients were flagged; 18 were already diagnosed for the disease.75/227 (33%) passed our internal review. 36 reports were returned to the GP. Feedback is currently available for 28/36:

	Reasonable possible diagnosis (Advanced for investigation)	Diagnosis has already been excluded	There is a clear alternative aetiology	Does not appear to be accurate	Patient no longer at the practice
# of patients EHR	9	6	10	2	1

Conclusions: This pilot demonstrates that implementation of such a toolis feasible at a population level with promising feedback from service users. The tool identified credible cases, subsequently referred for further investigation. Future work includes the ongoing follow up of flagged cases to ascertain if a final diagnosis is reached, and ongoing iterative performance-based validation of diagnostic algorithms.

W. Evans: A. Employment (full or part-time); Significant; Mendelian Ltd. D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); Modest; Takeda, Intrabio. **O. Buendia:** A. Employment (full or part-time); Significant; Mendelian Ltd. **C. Toal:** A. Employment (full or part-time); Modest; Mendelian Ltd. **P. Ravichandran:** A. Employment (full or part-time); Modest; Mendelian Ltd. **L. Menzies:** A. Employment (full or part-time); Modest; Mendelian Ltd.

P15.041.A Acoustic Droplet Ejection of Aqueous Solutions from an Acoustic Tube - Enabling qPCR

Maria Savino

Beckman Coulter France SAS, Villepinte, France.

Acoustic droplet ejection (ADE) is a fluid dispensing technology, whereby high-frequency sound waves are focused on the surface of a fluid to eject nano- and picoliter volumes with high accuracy and precision. We acoustically transferred 500 nL of gDNA (0.78 to 100 ng/µL) using an Echo Qualified Acoustic Tube and an Echo 655T into a qPCR plate. The achieved average accuracy and precision Coefficient of Variation (CV) values for transfers were measured to be <5%. This performance has enabled us to perform a quantitative real-time polymerase chain reaction gene expression assay build using the housekeeping gene, ß-actin. Assay Workflow• gDNA was sheared using a Covaris g-TUBE into 8kb fragment sizes.• Fragmented 8kb gDNA was used to generate an 8-point 2-fold standard curve.• 100 ng/µL, 50 ng/µL, 25 ng/µL, 12.5 ng/µL, 6.25 ng/µL, 3.13 ng/µL, 1.56 ng/µL, 0.78 ng/µL AnEcho Qualified Acoustic Tube was filled with 50 µL of each standard curve dilution point.• 0.5 µL gDNA was transferred using an Echo 655T to a 9.5 µL qPCR reaction. − 32 replicates per each standard curve dilution point• 0.5 µL gDNA was transferred using a hand held pipette (P2) to a 9.5 µL qPCR reaction as an experimental control.• gDNA was amplified using housekeeping gene, β-actin.

Conclusions• We present a new workflow to transfer sheared gDNA using Echo liquid handling technology.• The gDNA was transferred from Echo qualified tubes with comparable results to traditionalmethods.• We can further miniaturize the assay as the Echo 655T can accurately, precisely andreproducibly transfer 2.5 nL drops.

M. Savino: A. Employment (full or part-time); Significant; Beckman Coulter France SAS.

P15.042.B Biomek i7 Hybrid Automated Workstation Enables Automation of the Promega GoTaq Probe 2- Step RT-qPCR System

Bhagya Wijayawardena, ***Michael Hayes***

Beckman Coulter Life Sciences, Indianapolis, IN, USA.

Introduction: Reverse transcription quantitative PCR (RT-qPCR) is widely used for quantifying the amount of a specific RNA sequence in a sample. This method has proven extremely useful in a variety of workflows, including gene expression quantification and the diagnosis of infectious diseases, such as RNA viruses. Laboratory automation offers many advantages over manually preparing RT-qPCR assay plates, such as increased throughput and minimized likelihood of user-introduced error.

Materials and Methods: Here we describe automation of the Promega GoTaq Probe 2-Step RT-qPCR workflow using a Biomek Hybrid Automated Workstation.

Results: The automated method performed equivalently to manually performed assays, as 1 ng RNA input produced cycle threshold (CT) values of 21.60 ± 0.06 and 21.68 ± 0.08 for manual and automated methods, respectively. Additionally, the automated method maintained the wide dynamic range of the GoTaq kit. RNA input could be accurately quantitated over the entire range of inputs tested, from 10 ng to 3 pg, and when analyzed with linear regression, an R2 value of 0.9986 was observed.

Conclusions: Together the data presented here shows that the Biomek i7 Hybrid Automated Workstation can automate the RT-qPCR workflow, providing high quality results, while reducing user hands-on time and the possibility of user-introduced error.

B. Wijayawardena: A. Employment (full or part-time); Significant; Beckman Coulter Life Sciences. **M. Hayes:** None.

P15.043.C Enzymatic DNA Synthesis (EDS) enables decentralized and same-day access to DNA oligos critical for the study and detection of SARS-CoV-2

Benoit Derrien[1], *Chew Yee Ngan*[2], *Rahul Maurya*[2], *Sophie Romero*[1], *Nadège Tardieu*[1], *Henri Lachaize*[1], *Maëllys Kevin*[1], *Maryke Appel*[1], *Margarita L. Martinez-Fierro*[3], *Idalia Garza-Veloz*[3], *Alba S. H. Centeno-Ramirez*[4], *Xavier Godron*[1], *Chia-Lin Wei*[2]

[1]*DNA Script, LE KREMLIN BICETRE, France,* [2]*The Jackson Laboratory for Genomic Medicine, Farmington, CT, USA,* [3]*Autonomous University of Zacatecas, Zacatecas, Mexico,* [4]*Zacatecas State Health Services, Zacatecas, Mexico.*

The COVID-19 pandemic precipitated one of the most concentrated scientific efforts ever focused on the epidemiology, genomics, biochemistry and evolution of a single pathogen, and

on the development of diagnostic tests, treatments and vaccines. One unforeseen consequence of this effort was a global bottleneck in synthetic DNA supply, which currently relies on highly centralized phosphoramidite-based production and third-party logistics.

We have developed a novel enzymatic DNA synthesis (EDS) technology, which utilizes a highly engineered TdT enzyme, reversibly terminated nucleotides and a solid support. This enables same-day, on-demand DNA production with a benchtop "printer" — in a standard laboratory environment, requiring no specialized technical skills.

We have used the SYNTAX™ EDS System to produce oligos for SARS-CoV-2 LAMP, NGS and FISH assays. In this study, we report on the performance of EDS primers in the ARTIC network's hCoV-19 amplicon sequencing protocol (https://artic.network/ncov-2019). Libraries were prepared from two synthetic RNA control templates and five clinical samples with RT-qPCR Cq values ranging between 18.5 and 30.9. Comparable coverage (depth and uniformity) and 100% concordant SNP calling results were obtained with EDS primers and those obtained from commercial suppliers. Phylogenetic analysis of clinical isolates was performed in the context of >200 SARS-CoV-2 sequences submitted to public databases between December 2019 and June 2020.

Our study demonstrates that the EDS technology is mature enough to support genomics and life science applications, and holds the promise to revolutionize access to synthetic DNA — particularly in settings where in-house synthesis and fast iteration translates to tangible advantages.

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P15.044.D A fully automated workflow for SARS-CoV2 RNA detection

*Jethary Rader[1], Kinnari Watson[1], **Han Wei**[2]*

[1]Opentrons Labworks Inc, Brooklyn, NY, USA, [2]Beckman Coulter Life Science, Indianapolis, IN, USA.

Introduction: Viral pandemics present a significant threat to public health worldwide due to their vast and rapid spread of infection. The current outbreak of coronavirus (SARS-CoV-2) has highlighted a critical need to support high-throughput RNA detection. Here we demonstrate a fully automated workflow for SARS-CoV2 RNA detection with minimum manual processing times and provide users a robust, reproducible analysis solution during an outbreak.

Methods: Synthetic nasal matrix was spiked in with different concentrations of heat-inactivated SARS-CoV-2, and RNA was extracted using RNAdvance Viral kit on the Opentrons platform. Limit of detection (LoD) was quantified by RT-qPCR. In order to validate the RNA extracted with Opentrons can be sequenced, the viral RNA sequence was aligned to a known SARS-CoV-2 sequence.

Results: We observed that using the automated workflow, the LoD is 1 copy/uL for SARS-CoV-2 with a mean Ct 36. There is 100% detection for all the samples (N = 20) that spiked-in 1 copy/uL heat-inactivated virus. The alignment showed the samples to be very similar amongst each other and are similar when aligned to the Wuhan SARS-CoV-2 strain (2019-nCoV/USA-WA1/2020). The coverage is high among all the tested samples. The automated workflow can accommodate sample numbers from 8 to 96. To run 96 samples, about2.5 hours is needed with only 40 mins hands-on time, and each sample requires 10 tips.

Conclusions: In this proof of principle study, we demonstrate a robust and reliable automation workflow for viral RNA detection.

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P15.045.A Development of a novel, instrument-free, single-cell RNA sequencing technology (PIPseq) and its application to drug pathway discovery in lung cancer

*Iain Clark[1], Christopher D'Amato[2], Ahmad Osman[2], Sruti Pandey[2], Yi Xue[2], Aaron May-Zhang[2], Robert Meltzer[2], Sepehr Kiani[2], **Kristina Fontanez**[2], Adam Abate[3]*

[1]UC Berkeley, Berkeley, CA, USA, [2]Fluent BioSciences, Watertown, MA, USA, [3]University of California, San Francisco, San Francisco, CA, USA.

Introduction: Single-cell RNA sequencing (scRNA-Seq) has enabled unprecedented insight into the biology of individual cells across a broad range of discovery and disease -relevant applications. Traditional scRNA-Seq workflows have included single cell sorting into wells, co-capture of cells with barcoded beads using microfluidics, or in-cell combinatorial indexing. Fluent BioSciences has developed Pre-templated Instant Partitions (PIPs) to simultaneously segregate complex cell mixtures into partitions with barcoded template particles that can be easily processed for scRNA-Seq (PIPseq) without the need for complex instrumentation or microfluidic consumables. Here, we use PIPseq to bioinformatically discriminate the transcriptomes of Gefitinib resistant and sensitive cell lines after drug treatment.

Materials and Methods: To evaluate tyrosine kinase inhibitor effects on adenocarcinoma cellular transcriptomics, PC9 cells, H1975 cells, or a mixed population (9:1) of PC9 and H1975, cells were treated with Gefitinib and processed with PIPseq. UMAP analyses of the transcriptome were performed and overlapped for each experiment.

Results: We demonstrate drug-treatment dependent gene expression changes in lung adenocarcinoma cells treated with the tyrosine kinase inhibitor Gefitinib. The resulting cell expression profiles clearly segregate by treatment condition in UMAP projections indicating PIPseq's ability to faithfully detect responses to drug exposure.

Conclusion: These studies establish the efficacy of PIPseq for single-cell transcriptomic analysis across multiple drug treatment conditions. The simple PIPseq workflow is optimized for comparing multiple sample treatment conditions in a single controlled experiment. With minimal upfront cost of implementation, PIPseq is easily implemented in any molecular research lab, and democratizes the accessibility of scRNA-Seq across many applications.

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P15.046.B Application of a novel instrument-free and microfluidics-free single-cell analysis technology (PIPseq) that is well suited for viral applications in resource constrained laboratories

*Jacquelyn Turcinovic[1], John Connor[1], Aaron May-Zhang[2], Ahmad Osman[2], **Robert Meltzer**[2], Sepehr Kiani[2], Kristina Fontanez[2]*

[1]Boston University, Boston, MA, USA, [2]Fluent BioSciences, Watertown, MA, USA.

Introduction: Single cell RNA sequencing (scRNA-Seq) has made profound impacts in the study of cellular and molecular diversity in complex tissues. In the study of virology, this resolution of single-cell transcriptional changes in response to viral infection provides valuable insight into the mechanisms of infection and host response.

Materials and Methods: Current scRNA-Seq methods are not easily adopted in the virology lab as they are expensive, require complex instrumentation and consumables, and hence can be challenging to implement in a laboratory with limited resources and accessibility. Fluent BioSciences has developed a novel scRNA-Seq approach with Pre-templated Instant partitions (PIPseq) that enables the analysis of thousands of cells without requiring complex instrumentation and consumables. The small format and convenient workflow, with the lack of instrumentation, allows PIPseq to be easily implemented in high-containment laboratories.

Results: Using a GFP-expressing control virus, we have demonstrated simultaneous capture and barcoding of viral and cellular transcriptomes, identified cellular gene expression shifts in response to viral infection, and identified gene expression responses in non-infected cells from low MOI infected samples compared to mock controls. Furthermore, we have demonstrated that the PIPseq protocol is effective at inactivating residual virus in sequencing library samples, thus enabling convenient sample post-processing outside of the virology laboratory.

Conclusion: PIPseq is an effective method to study viral-host interactions at a single-cell resolution. The ongoing COVID-19 pandemic exemplifies the need for new tools and methods to elucidate the mechanisms of viral infection, pathogen-host responses, and diversity in cellular responses to infection.

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P15.047.C Simultaneous profiling of human TCR and BCR rearrangements at the DNA level

***Alexander Y. Komkov**[1,2], Anna Miroshnichenkova[2,1], Anastasia Smirnova[1,3], Valeria Tkachenko[1,4], Yuri Lebedev[1], Dmitry Chudakov[1], Ilgar Mamedov[1,2]*

[1]Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Moscow, Russian Federation, [2]Dmitry Rogachev National Medical Research Center Of Pediatric Hematology, Oncology and Immunology, Moscow, Russian Federation, [3]Skolkovo Institute of Science and Technology, Moscow, Russian Federation, [4]Moscow Institute of Physics and Technology, Moscow, Russian Federation.

Introduction: Immune receptors repertoire (TCR/BCR) profiling is a powerful source of basic and applied insights in immunogenetics. The most sensitive and reliable method for TCR/BCR profiling is DNA based target multiplex PCR in combination with high-throughput sequencing. The most existing multiplex-based methods enable to analyze TRA, TRB, TRD, TRG, IGH, IGK and IGL loci but only separately. It substantially limits the applicability of this approach for the analysis of samples with restricted target DNA amounts. Here we resolve this issue by proposing the first technology for joint detection of all TCR/BCR rearrangements in a single multiplex PCR reaction.

Material and methods: Sequences of TCR/BCR V, D and J-genes for primers design were downloaded from IMGT database. Test samples of genomic DNA were extracted from PBMC of healthy volunteers. Each mixed TCR/BCR repertoire was obtained by multiplex PCR with subsequent Illumina sequencing and data analysis using MiXCR software.

Results: Multiplex primers were designed using k-mer approach. Artifacts were removed by primers redesign after each iteration of library sequencing and analysis. Primer concentrations in the resulting set were normalized using iROAR software. The final multiplex system (exclusively distributed by MiLaboratory) was successfully tested on 20 DNA samples from PBMC with series dilutions: 50000, 5000, 500 and 50 genome equivalents.

Conclusion: The developed system decreases the labor intensity of TCR/BCR analysis and it is indispensable for TCR/BCR profiling of small populations of T/B lymphocytes, tumor infiltrated lymphocytes and residual leukemic cells. This work was supported by RSF grant №20-75-10091 and RFBR grant №20-015-00462.

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P15.048.D Semi-targeted sequencing of fusion transcripts in prostate cancer

Ugne Drazdauskiene[1], *Zana Kapustina*[1,2], *Justina Medziune*[1,3], *Monika Gasiuniene*[1], *Ruta Sindikeviciene*[1], *Varvara Dubovskaja*[1], *Rasa Sabaliauskaite*[4], *Arvydas Lubys*[1]

[1]*Thermo Fisher Scientific Baltics UAB, Vilnius, Lithuania,* [2]*Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania,* [3]*Institute of Chemistry, Faculty of Chemistry and Geosciences, Vilnius University, Vilnius, Lithuania,* [4]*National Cancer Institute, Vilnius, Lithuania.*

Introduction: Chromosomal rearrangements are the most common genetic changes in cancer genomes and they often lead to the formation of gene fusions which may be transcribed into fusion transcripts. Current detection methods rely on PCR- or hybridization-based techniques that do not allow the detection of novel fusion breakpoints. To overcome these challenges, we developed a new rapid and accurate identification method, termed fusion sequencing via terminator-assisted synthesis (FTAS-seq), for high-throughput gene fusion profiling.

Materials and Methods: We developed oligonucleotide-tethered dideoxynucleotides (OTDDNs) to capture unknown sequences downstream of the target site. Modified OTDDNs, upon random incorporation during primer extension reaction, create DNA fragments of a desired average length, with simultaneous labeling of a corresponding DNA strand with sequencing adapter. Oligonucleotide modification then serves as a priming site for subsequent synthesis of cDNA strand. We prepared semi-targeted RNA-seq libraries from prostate cancer cell line RNA and clinical samples and sequenced them.

Results: We applied FTAS-seq to study *TMPRSS2-ERG* fusion transcripts in prostate cancer cell line NCI-H660 to validate the approach. Also, we applied the FTAS-seq to evaluate *TMPRSS2-ERG* expression levels and variety in clinical samples.

Conclusions: Additionally, this method can be used to analyze genomic rearrangements, such as *ALK* gene fusions in non-small cell lung cancer specimens. FTAS-seq could be successfully applied for the known and novel breakpoints detection. It is a good alternative to amplicon sequencing as it has greater discovery potential at the same level of cost-effectiveness.

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P15.049.A Integration of genomics and transcriptomics to identify DNA damage defects in PID patients prone to cancer

Lynn Backers[1,2,3], *Bram Parton*[1,2,3], *Mattias Van Heetvelde*[1,2,3], *Marieke De Bruyne*[1,2], *Kim De Leeneer*[1,2,3], *Anne Vral*[4], *Filomeen Haerynck*[5], *Kathleen B. M. Claes*[1,2,3]

[1]*Ghent University, Department of Biomolecular Medicine, Gent, Belgium,* [2]*Center for Medical Genetics Ghent, Ghent, Belgium,* [3]*Cancer Research Institute Ghent (CRIG), Ghent, Belgium,* [4]*Ghent University, Department of Human Structure and Repair, Gent, Belgium,* [5]*Ghent University, Department of Internal Medicine and Pediatrics, Gent, Belgium.*

Introduction: Primary immunodeficiencies (PIDs) are a heterogeneous group of disorders caused by genetically determined defects of the immune system, predisposing to life-threatening complications such as severe and recurrent infections, auto-immunity and malignancies. A subset of PIDs is caused by pathogenic germline variants in DNA repair genes. As a consequence these patients are radiosensitive and present with increased cancer risk. Since PID patients are exposed to radiation for several reasons (bone marrow transplantation, radiotherapy, diagnostic imaging), identification of the genetic defect is important for risk stratification and improved therapeutic management. As in only 5-20% of the patients pathogenic variants are found in currently known PID genes, we hypothesize that defects in additional DNA damage response genes may be involved in PID patients prone to malignancies.

Materials and Methods: WES data is analyzed by a DNA damage response panel consisting of +/- 1230 genes. Extensive filtering results in a long list of variants of unknown clinical significance (VUS). To facilitate variant prioritization we are complementing WES with transcriptomics on RNA extracted from short term lymphocyte cultures. We search for expression and splicing outliers in the transcriptome.

Results: Using this approach we identified a homozygous intronic variant, outside the canonical splice sites, in a DNA repair gene not previously linked to PID. RNA-seq revealed out-of-frame exon skipping. Further functional validations to establish a link with the phenotype are ongoing.

Conclusions: These first findings encourage the implementation of transcriptomics in the workup of PID patients to improve diagnosis and patient management.

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P15.050.B Unraveling the genetic thread of rare disorders by exome sequencing

Petroula Gerasimou[1], *Andri Miltiadous*[1], *Ioannis Kyprianou*[1], *Jianxiang Chi*[1], *Violeta Anastasiadou*[2], *George Tanteles*[3], *Paul Costeas*[1]

[1]*Karaiskakio Foundation, Nicosia, Cyprus,* [2]*Department of Clinical Genetics, Archbishop Makarios III Medical Centre, Nicosia, Cyprus,* [3]*Department of Clinical Genetics, Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus.*

Over the last few years, Next Generation Sequencing has become an essential tool in laboratory practice due to its high throughput, analytic accuracy, and potential cost-effectiveness. Exome sequencing (ES) has revolutionized diagnostic procedures in medical genetics. Previous diagnostic approaches, involved a combination of cytogenetic and metabolic methods as well as targeted gene sequencing with a success rate of identifying the genetic cause in around 20-30% of undiagnosed patients. This percentage of diagnostic yield in undiagnosed cases rises to 25-50% with ES. In addition, the true impact of ES is evident from the opportunity to end patient's diagnosis anxiety, offer family prognosis or timely diagnosis and family planning, proper care protocols, and psychological and emotional well-being. In collaboration with medical geneticists and oncologists, the molecular laboratory of Karaiskakio Foundation (KF), offers whole exome sequencing (ISO15980 accredited since 2019) using SureSelect Human All Exon V7 (Agilent CA, USA) to patients with hereditary cancer or rare syndrome suspicion that were otherwise molecularly undiagnosed. With this latest technology in molecular diagnostics, KF have assisted in the molecularly diagnosis

of more than 150 patients with genetic diseases to date, 99 of whom would not have had this opportunity through other conventional methods. 51 cases have been tested and diagnosed for Cancer Diseases, Overgrowth syndromes and Rasopathies and Pigmentary disorders, while another 40 have been confirmed for rare genetic syndromes such as Chanarin Dorfmann; Central core disease; Spinocerebellar ataxia type 29; Dehydrated hereditary stomatocytosis; Cornelia De Lange; Joubert Syndrome 23; Ehlers-Danlos, among other.

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P15.051.C Whole genome sequencing in apparently balanced de novo chromosomal translocations in 10 patients with malformations and/or intellectual disability

Irene Valenzuela Palafoll[1], *Alejandro Romera-Lopez*[2], *Anna M. Cueto-Gonzalez*[1], *Alfonso Andujar*[3], *Dan Diego*[4], *Sonia Santillan*[5], *Guillermo Marco*[6], *Christian Moya*[7], *elena garcia-arumi*[1], *Ivon cusco*[1], *Eduardo F Tizzano*[1]

[1]*Hospital Vall d'Hebron, Barcelona, Spain,* [2]*Sistemas Genómicos -Ascires, Valencia, Spain,* [3]*Sistemas genómicos, valencia, Spain,* [4]*sistemas genómicos, Valencia, Spain,* [5]*Sistemas genómicos, Valencia, Spain,* [6]*Sistemas Genómicos, Valencia, Spain,* [7]*Sistemas genómicos- Ascires, Valecia, Spain.*

Balanced chromosomal abnormalities (BCA) are a class of structural variation involving rearrangement of chromosome organization without a concomitant large gain or loss of DNA. A five-fold increase in the prevalence of karyotype BCAs has been reported among subjects with neurodevelopmental disorders. Genome technologies can efficiently reveal BCA breakpoints at nucleotide resolution; however, a limited number of BCAs have been evaluated to date. We used whole genome sequencing (WGS) to characterize breakpoints of BCAs at the molecular level in 10 patients with intellectual disability and/or congenital anomalies. Breakpoints were characterised by a paired-end low depth WGS and were successfully mapped in all of them. In four cases mapping of breakpoints clearly identified the molecular cause of the phenotype. Two of these patients had a disruption of regulatory elements: one in *MEF2C* TAD and the other in *SOX9*. In the remaining two patients one had an intragenic disruption of *GRIN2B*and the other had intragenic disruption of *EHMT1*. Another patient without prior NGS studies showed a SNV[X1] in ANKRD11 that was identified as responsible of the phenotype. In the remaining five patients even though their breakpoints were successfully mapped, no molecular explanation was found to explain their respective phenotypes. In our experience, WGS allowed precisely mapping breakpoints in our patients, constituting an unparalleled opportunity to improve our understanding of genes involved in genetically complex disorders. Characterization of BCAs at nucleotide resolution offers new insights into their mechanisms of formation and a yield of potentially novel genes associated with human development

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P16 Diagnostic Improvements and Quality Control

P16.001.D Fusion gene detection by RNA-Seq complements AML diagnostics and identifies recurring *NRIP1-MIR99AHG* rearrangements

Paul Kerbs[1,2,3], *Sebastian Vosberg*[1,2,3], *Stefan Krebs*[4], *Alexander Graf*[4], *Helmut Blum*[4], *Anja Swoboda*[1], *Aarif M. Nazeer Batcha*[5], *Ulrich Mansmann*[5], *Dirk Metzler*[6], *Caroline A. Heckman*[7], *Tobias Herold*[1,2,3], *Philipp A. Greif*[1,2,3]

[1]*Department of Medicine III, University Hospital, LMU Munich, Munich, Germany,* [2]*German Cancer Consortium (DKTK), partner site Munich, Munich, Germany,* [3]*German Cancer Research Center (DKFZ), Heidelberg, Munich, Germany,* [4]*Laboratory for Functional Genome Analysis (LAFUGA), Gene Center, LMU Munich, Munich, Germany,* [5]*Department of Medical Data Processing, Biometry and Epidemiology, LMU Munich, Munich, Germany,* [6]*Division of Evolutionary Biology, Faculty of Biology, LMU Munich, Planegg-Martinsried, Germany,* [7]*Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Helsinki, Finland.*

Identification of fusion genes in clinical routine is mostly based on cytogenetics and targeted molecular genetics, such as metaphase karyotyping, FISH and RT-PCR. However, sequencing technologies like RNA-seq are becoming more important in clinical routine. To evaluate the performance of fusion gene detection by RNA-seq compared to standard diagnostic techniques, we analyzed 806 RNA-seq samples from AML patients using two state-of-the-art software tools, namely Arriba and FusionCatcher. RNA-seq detected 90% of fusion events that were reported by routine with high evidence, while samples in which RNA-seq failed to detect fusion genes had overall lower and inhomogeneous sequence coverage. Based on properties of known and unknown fusion events, we developed a workflow with integrated filtering strategies for the identification of robust fusion gene candidates by RNA-seq. Thereby, we detected known recurrent fusion events in 26 cases that were not reported by routine. Moreover, we identified 157 novel robust fusion gene candidates. Finally, we detected the novel recurrent fusion gene *NRIP1-MIR99AHG* resulting from inv(21)(q11.2;q21.1) in nine patients (1.1%) and *LTN1-MX1* resulting from inv(21)(q21.3;q22.3) in two patients (0.25%). We demonstrated that *NRIP1-MIR99AHG* results in overexpression of the 3′ region of *MIR99AHG* and the disruption of the tricistronic miRNA cluster miR-99a/let-7c/miR-125b-2. Interestingly, upregulation of *MIR99AHG* and deregulation of the miRNA cluster, residing in the *MIR99AHG* locus, are known mechanism of leukemogenesis in acute megakaryoblastic leukemia. Our findings demonstrate that RNA-seq has a strong potential to improve the systematic detection of fusion genes in clinical applications and provides a valuable tool for fusion gene discovery.

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P16.002.A The effect of the high resolution chromosomal microarray analysis on diagnosis of single gene disorders

Ahmet Cevdet Ceylan[1,2], *Vahap Topçu*[2], ***Esra Habiloglu***[2], *Gülay Güleç Ceylan*[1,2], *Nur C. Semerci Gündüz*[1,2]

[1]*ANKARA YILDIRIM BEYAZIT UNIVERSITY, Ankara, Turkey,* [2]*Ankara City Hospital, Medical Genetics Department, Ankara, Turkey.*

Introduction: Chromosomal microarray analysis is a first-stage test that is used for the diagnosis of intellectual disability and global developmental delay. Chromosomal microarray analysis (CMA) can detect well-known microdeletion syndromes. It also contributes to the diagnosis of single gene disordersif high resolution array is used.

Methods: Illumina 850k® chips were used to perform CMA in 2000 affected individual for different clinical reasons at Ankara City Hospital in 2019-2020. Data analysis was performed using

BlueFuse Multi 4.5 software. Only changes associated with the patient's phenotype below 500 kb were re-analysed.

Results: 34 pathogenic variants were detected out of 2000 patients. 13 of them were homozygous deletion which are Juvenile parkinsonism-2(3),Autosomal recessive(AR) Deafness-2 (2),Rotor(2),AR Spinocerebellar ataxia-18, AR Hyper-IgE,Infantile-onset limb and orofacial dyskinesia, Joubert, Hair-Brain, Pitt-Hopkinslike syndromes. 19 detected variants were heterozygous or hemizygous CNVs' associated with Marfan(6), Duchenne muscular dystrophy(3),Landau-Kleffner(3), Phelan-McDermid(2), Tuberous sclerosis-2, Hyperekplexia, Langer mesomelic dysplasia,Growth hormone deficiency, GLUT1 deficiency syndromes. Two of them were heterozygous duplication which results Simpson-Golabi-Behmel and Xq28 Duplication Syndromes.

Discussion: CMA is the gold standard method for detecting CNVs. As higher resolution scanning chips are used, smaller CNVs can be detected. Although the percentage of diagnosis of CMA varies according to the disease, different rates between 10-25% have been reported. In our study, we evaluated the pathogenic CNVs below 500 kb and revealed that the 1.7% diagnosed rate more. Especially, detection of some deletions as homozygous and diagnosis of DMD before specific symptoms reveals the importance of CMA.

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P16.003.B Clinical Genetics Assessment Tool (CGAT): A novel and simple approach for pediatricians to suspect genetic disorders

Aitor López-González[1], *David Ferri*[1], *Dídac Casas-Alba*[1], *Daniel Cuadras*[2], *Francesc Palau*[1], *Antonio Martínez-Monseny*[1]

[1]*Hospital Sant Joan de Déu, Esplugues de Llobregat, Barcelona, Spain,* [2]*Fundació Sant Joan de Déu, Esplugues de Llobregat, Barcelona, Spain.*

Introduction: The identification of children with a genetic disorder is a challenge for pediatricians and clear criteria for referral to Clinical Genetics Units are still lacking. We aim to elaborate a simple clinical tool to identify patients with a greater probability of having a genetic disorder.

Methods: A single-center descriptive and retrospective study was carried out based on electronic medical records. Patients under 18 years of age followed up in our Clinical Genetics Unit from June 2018 to January 2019 were selected.

Results: From a total of 450 patients, 304 were included. The variables most associated with diagnosis were: diagnostic orientation in the referral ($p = 0.001$), multiple congenital anomalies (MCA) ($p < 0.001$), short stature (SS) ($p < 0.001$), intellectual disability (ID) ($p = 0.001$), craniofacial dysmorphic features ($p = 0.006$), ectodermal ($p = 0.004$), onco-hematological ($p = 0.034$), ophthalmological anomalies ($p = 0.006$) and failure-to-thrive ($p = 0.034$). A ROC curve was created (AUC 0.73), with 84.4% sensitivity and 44.7% specificity to reach a genetic diagnosis if 2 or more of these variables were present. ORs of reaching a genetic diagnosis for patients with SS, ID and MCA were 11.46 ($p = 0.003$); SS and ID 5.57 ($p < 0.001$); SS and MCA 9.18 ($p < 0.001$); and ID and MCA 31.67 ($p < 0.001$).

Conclusions: This is one of the first studies exploring the predictive value of clinical variables on reaching a genetic diagnosis. The pediatrician's knowledge on Clinical Genetics appears to have a key impact on it. SS, ID and MCA may form the sides of a triangle constituting a simple clinical genetics assessment tool (CGAT) for referral to Clinical Genetics Unit.

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P16.004.C Copy number variants in a large cohort analysed with whole-exome sequencing: lessons for genetic diagnosis

Fátima Lopes[1,2], *Alexandra M. Lopes*[1,2], *Paulo Silva*[1,2], *Susana Sousa*[1,2], *Sara Morais*[1,2], *Joana Sá*[1,2], *Ana Filipa Brandão*[1,2], *Ana Lopes*[1,2], *Rita Bastos-Ferreira*[1,2], *João Parente Freixo*[1,2], *Jorge Sequeiros*[1,2], *Jorge Oliveira*[1,2]

[1]*CGPP-IBMC – Centro de Genética Preditiva e Preventiva, Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal,* [2]*i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal.*

Whole-exome sequencing (WES) enables the simultaneous analysis of all coding regions of the human genome. Although WES started by targeting primarily the detection of single nucleotide variants and small insertion/deletions, bioinformatics tools have been developed to help detecting copy number variants (CNVs). The aim of this work was to evaluate the efficacy of read depth-based CNV detection in routine diagnostics. We performed WES in 3,319 consecutive samples (2016 through 2020), referred for genetic testing in a wide variety of diseases. Exomes were captured with Agilent's SureSelect Human AllExon ($n = 2819$) or Twist's Human Core Exome Kit ($n = 500$) and sequenced on Illumina platform HiSeq4000 or NovaSeq. CNVs detection by read depth-based analysis was performed with VarSeq (Golden Helix). CNVs considered likely to contribute to patients' phenotype were confirmed either by qPCR or MLPA. From 3,319 patients tested, 152 clinically relevant CNVs (36 duplications, 116 deletions) were reported, a diagnostic yield of 4.6%. Subdividing by panel or disease group, yield was 7.6% for an ocular diseases' panel, 6.8% for neurodevelopment spectrum disorders, 4.1% with clinical exome, 4.0% with neuroexome, 4.3% for WES trios, 3.3% in neuromuscular diseases, 2.3% in movement disorders, 2.1% in vascular diseases and 1.8% in neuropathies.The inclusion of read depth-based CNV detection from NGS data in routine bioinformatics pipelines is a cost-effective add-on for diagnostic laboratories. Nevertheless, given the high rate of false positives, this strategy requires confirmation by another methodology of all clinically relevant CNVs detected.

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P16.005.D Congenital anomalies and genetic disorders in neonates and infants: a single-center observational cohort study

Abderrahim Marouane[1], *Richelle A. C. M. Olde Keizer*[2], *Geert W. J. Frederix*[2], *Lisenka E. L. M. Vissers*[1], *Willem P. de Boode*[3], *Wendy A. G. van Zelst-Stams*[1]

[1]*Department of Human Genetics, Radboud University Medical Center, Nijmegen, Netherlands,* [2]*Department of Health Sciences and Primary Care, University Medical Center, Utrecht, Netherlands,* [3]*Department of Neonatology, Radboud University Medical Center, Amalia Children's Hospital, Nijmegen, Netherlands.*

Objective: Neonates with genetic disorders or congenital anomalies (CA) contribute considerably to morbidity and mortality

in neonatal intensive care units (NICUs). In many of these neonates, an underlying genetic condition is identified throughout life. The objective of this study is to study the prevalence of genetic disorders in an academic level IV NICU by identifying and describing assessed genetic disorders, used genetic testing methodologies, and both timing of or time to diagnosis.

Study design: This is a retrospective analysis of infants admitted to the NICU of the Radboud University Medical Center between 1 October 2013 and 31 October 2015. Data were collected until infants reached at least two years of age.

Results: 13% (194/1,444) of the patients were genetically tested. Approximately one-third (32%; 461/1,444) of our cohort had a CA. 37% (72/194) had a laboratory-confirmed genetic diagnosis. In 53% (38/72) the diagnosis was made post-neonatally (median age = 209 days) using assays including exome sequencing. 63% (291/461) of the patients with CA, however, never received genetic testing, despite being clinically similar those who did.

Conclusions: Genetic disorders were suspected in 13% of the cohort, but only confirmed in 5%. Most received their genetic diagnosis in the post-neonatal period. Extrapolation of the diagnostic yield suggests that up to 6% of our cohort may have remained genetically undiagnosed. Our data show the need to improve genetic care in the NICU for more inclusive, earlier and faster genetic diagnosis to enable tailored management.

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P16.006.A Clinical impact in 120 undiagnosed cases of extending single proband WES to trio analysis

Monica Martinez-Garcia[1], *Gema Gordo*[1], *Julia Gonzalez-Rincon*[1], *Celia Rodriguez-Solera*[1], *Irene Diez*[1], *Angel Carro*[1], *Iker Sanchez-Navarro*[1], *Eva Barroso*[1], *Guillermo Martin-Serrano*[1], *Marta Hervas*[1], *Andrea Fuente-Revenga*[1], *Pablo Solar*[1], *Marta Carcajona*[1], *Noelia Sánchez-Bolivar*[1], *David Rodriguez*[1], *Miguel Angel Grillo*[1], *Rocio Jadraque*[2], *Carmen Carrascosa*[3], *Ana Fontalba*[4], *Asuncion Villanueva*[5], *Maria Dolores Miramar*[6], *Ana Rodriguez-Valle*[6], *Silvia Izquierdo*[6], *Ricardo Gonzalez-Tarancon*[6], *Paolo Maietta*[1], *Sara Alvarez*[1]

[1]*Unidad de secuenciación, NIMGenetics S.L., Madrid, Spain, Madrid, Spain,* [2]*Hospital General Universitario Alicante, Alicante, Spain,* [3]*Complejo Hospitalario Unviersitario de Albacete, Albacete, Spain,* [4]*Hospital Universitario Marques de Valdecilla, Santander, Spain,* [5]*Hospital Universitario Virgen del Rocio, Sevilla, Spain,* [6]*Hospital Universitario Miguel Servet, Zaragoza, Spain.*

Background: Exome trio analysis is an effective strategy to identify causal variants responsible for rare genetic disorders. The goal of this study was to identify the effectiveness of a trio analysis to establish the genotype-phenotype correlation in patients with a previous whole exome sequencing analysis (WES).

Methods: A cohort of 120 probands, with a median age of 5 y.o, primarily studied by targeted or clinical exome, and most of them without a clear clinical diagnosis (mainly, neurodevelopmental disorders) was extended to trio analysis. WES was performed using Twist Bioscience technology with NovaSeq 6000 System. Sequencing reads were analyzed using DRAGEN BioIT Platform and an in-house pipeline.

Results: The molecular diagnosis was obtained in over 30% of the extended studies. Depending on the initial analysis, a new diagnostic variant was identified, or the segregation pattern pointed to a previously identified variant. These genomic changes were mainly de novo missense novel variants (42%), and most of them were in genes with a known OMIM association. Many variants with mild overlapping phenotype were dismissed. Chromosomes 1 and X were the most representative ones presenting causal variants located in genes involved in cell differentiation, chromatin remodeling and DNA replication.

Conclusion: The extension of single proband WES to trio analysis, HPOs prioritization, and recurrent updating of databases are essential to establish the definite diagnosis by discriminating causal variants among overlapping pathologies, discovering new genes, and changing variants categorization. We show the advantages of using a stepwise approach to WES diagnosis in clinical institutions.

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P16.007.B WINGS: Wales Infants and ChildreNs Genome Service. Rapid and accurate clinical analysis of variants from Whole Genome Sequencing assays using a user friendly web application

Joseph Halstead

All Wales Medical Genomics Service, Cardiff, United Kingdom.

Introduction: In 2020 the All Wales Medical Genomics Service (AWMGS) implemented a rapid Whole Genome Sequencing (WGS) service for critically ill children. The original in house bioinformatics solution for filtering variants had several limitations. 1) The results were displayed in a text file which limited the annotations which that could be displayed. 2) Scientists analysing the case could not modify filtering parameters to investigate cases in more detail. 3) Applying custom virtual gene panels required trained bioinformaticians. 4) There was no method for calculating in house variant frequency. To overcome these limitations AWMGS has developed a web application for analysing WGS cases.

Methods: The application has been developed using a combination of the Python based web framework Django and the SQL database Postgres. The software is accessible to users via a modern web browser. WGS Trios from 11 previous cases were uploaded to the software and the results analysed.

Results: The web application successfully replicated the results from the previous cases. The software was able to process each case in less than 15 minutes when running on a desktop computer. The software successfully identified variants which occurred in separate samples helping to identify assay specific sequencing artefacts. Users were able to rapidly investigate the variants in specific genes without needing bioinformatics support.

Discussion: The new web application gives scientists analysing WGS cases greater flexibility whilst maintaining the sensitivity of the original bioinformatics solutions. Future work will integrate other AWMGS NGS assays into the software.

J. Halstead: None.

P16.009.D Offering exome-wide NGS analysis within a diagnostic time-frame: promise or pitfall

Kristin M. Abbott

University Medical Center Groningen, Groningen, Netherlands.

Whole exome sequencing with panels involving only genes with known disease interactions are routinely applied in the diagnostic testing. Exome-wide genetic analyses, considering all protein coding genes, could increase diagnostic yield, but at the cost of turn-around times. Still, the relatively high number of unsolved cases after routine NGS panel diagnostics prompted us to develop a structured analysis approach to allow exome-wide analysis within a diagnostic time-frame. Our analysis strategy is based on 4 key principles: 1) it is only offered as follow-up NGS testing after WES-based gene panel diagnostics is negative, 2) it is offered as a trio-analysis with healthy parents, 3) a specific variant/gene filter strategy based on inheritance is used and 4) a consequent analysis strategy with stepwise decision points and scheduled consultation moments is also used. We have completed approximately 340 exome-wide analyses over the last three years, averaging approximately 10 cases per month with a TAT of 42 days. Thirty percent of our unsolved cases receive a result based on literature, animal studies and other information. Results include relevant genes outside the previously requested panels, and new relevant information not available during the original analysis. New candidate genes have also been identified. Future follow-up of literature and databases will determine whether these candidate genes prove to be valid, increasing diagnostic yield even more. In conclusion, with our systematic approach, we achieved higher diagnostic yield within a 42 day TAT, thus showing that exome wide screening is feasible for implementation in a diagnostic setting.

K.M. Abbott: None.

P16.010.A Belgian guidelines for the frequency of participation of the Medical Centers of Human Genetics to External Quality Assessment schemes for analyses focused on rare diseases

josephine lantoine[1], *Anne Brysse*[2], *Vinciane Dideberg*[2], *Kathleen B. M. Claes*[3], *Sofie Symoens*[3], *Wim Coucke*[1], *Valérie Benoit*[4], *Sonia Rombout*[4], *Martine De Rycke*[5], *Sara Seneca*[5], *Lut Van Laer*[6], *Wim Wuyts*[6], *Anniek Corveleyn*[7], *Kris Van Den Bogaert*[7], *Catherine Rydlewski*[8], *Françoise Wilkin*[8], *Marie Ravoet*[9], *Elodie Fastré*[9], *Arnaud Capron*[1], *Nathalie M. Vandevelde*[1]

[1]*Department of Quality of Laboratories, Sciensano, Brussels, Belgium,* [2]*Center of Human Genetics, CHU of Liege, University of Liege, Liège, Belgium,* [3]*Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium,* [4]*Center of Human Genetics, Institut de Pathologie et de Génétique, Gosselies, Belgium,* [5]*Center for Medical Genetics, Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel, Brussels, Belgium,* [6]*Center of Medical Genetics, Antwerp University Hospital and University of Antwerp, Edegem, Belgium,* [7]*Center for Human Genetics, University Hospitals Leuven, Leuven, Belgium,* [8]*Center of Human Genetics, Hôpital Erasme, Université Libre de Bruxelles, Anderlecht, Belgium,* [9]*Center for Human Genetics, Cliniques universitaires Saint-Luc, Université catholique de Louvain, Brussels, Belgium.*

Aims: The participation to external quality assessments (EQAs) is required for the ISO15189 accreditation of the Belgian Medical Centers of Human Genetics. However, no directives on the minimal frequency of participation to genetic EQAs exist and European recommendations in this field are heterogeneous. This potentially impacts healthcare quality.

Method: In order to address this lack, genetic EQAs offered by accredited providers and focused on analyses used for rare diseases' diagnosis were analyzed by a working group in order to propose minimal frequencies of participation to EQA and recommendations for dealing with poor performances and change management.

Results: Our guidelines offer a decisional algorithm built on 3 key principles: (i) the recommended annual assessment of all techniques and technological platforms, if possible through EQAs covering the technique, genotyping and interpretation, (ii) the triennial assessment of the genotyping and interpretation of specific germline mutations and pharmacogenomics analyses, (iii) the documentation of actions undertaken in the case of poor performances and the participation to a quality control the following year. Besides, these guidelines demonstrate the cost-effectiveness of the rationalization of the frequency of participation to these quality controls.

Conclusions: These guidelines are built on the analysis of a large panel of quality controls and data collected from the Belgian centers. However, they are totally applicable to other countries and will facilitate and improve the quality management of the medical centers of human genetics.

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P16.011.B DNA re-analysis of highly suspected FAP patients by CZECANCA NGS panel

Petra Slavíková, Jitka Štekrová, Petra Kleiblová, Petra Zemánková, ***Markéta Urbanová***

Institute of Biology and Medical Genetics of 1st Medical Faculty Charles University and General Teaching Hospital, Prague, Prague, Czech Republic.

Introduction: Familial adenomatous polyposis (FAP) is a cancer predisposition syndrome caused by germline mutations in tumor suppressor gene *APC*, which is inherited in autosomal dominant manner. The aim of this study was to re-analyze old DNA samples of highly suspected patients whose diagnosis previously failed to be confirmed by at that time commonly used methods of molecular diagnostics.

Materials and Methods: Next generation sequencing was performed on patients' DNA isolated from peripheral blood lymphocytes (N = 78, samples from years 1993-2004) using gene panel CZECANCA (Czech Cancer Panel for Clinical Application, version 1.2) that covers 226 genes and where *APC* gene encompasses also the promoter region that was proven to be important in FAP diagnostics.

Results: In our cohort, 18 % (14/78) of re-analyzed patients carry pathogenic variant in the *APC* gene (class 4 and 5). The most common deleterious type of mutation in *APC*, frameshift and nonsense variants, were found in 5 and 2 patients respectively. We have also detected 2 patients with deletions in the promoter 1B region. In another 10 % of patients we detected pathogenic variants in other genes associated with colorectal cancer predisposition, which led to reevaluation of their diagnosis. We have recorded variants of unknown clinical significance among 25 % of patients.

Conclusions: NGS gene panel CZECANCA proved to be a valuable tool that brought improvement in diagnostic yield of FAP patients in our cohort. Based on the obtained data, we were able

to confirm or reevaluate diagnosis. Funding: Charles University Research Fund Progress Q28/LF1.

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P16.014.A Validations of genotyping array analysis as a diagnostic method in medical genetics

Martina Witsch-Baumgartner[1], *Silja Burkhard*[1], *Beatrix Mühlegger*[1], *Rebekka Gröbner*[1], *Gunda Schwaninger*[1], *Peter Kirchmeier*[2], *Johannes Zschocke*[1]

[1]*Medical University Innsbruck, Innsbruck, Austria,* [2]*JSI medical systems GmbH, Ettenheim, Germany.*

Introduction: We evaluated the potential of standard genotyping arrays for diagnostic purposes in a clinical setting.

Methods: Genotyping results with the Global Screening Array (Illumina GSA-24 v.2.0 or v3.0+Multi-Disease content) were assessed in 530 independently sequenced diagnostic DNA samples for 29 clinical indications involving 55 different genes and 129 different variants. Data analysis and variant reporting was performed with the SeqArray software (JSI, Germany).

ID="Par5422">**Results:** Analysis of 122/129 not previously GSA-validated variants fulfilled quality criteria in all samples and showed correct identification. 8/129 variants failed quality thresholds but were not genotyped as absent either. Assay sensitivity thus was 94% but there were no true false negatives. One gene (*LDLR*) showed three false positive results, possibly linked to the high number of *LDLR* variants on the array; there were no false positives in the other genes studied. Genotyping was fully accurate for specific target variants (e.g. genes *F5, F2, ALDOB, DPYD, LCT*). Clinical sensitivity for monogenic diseases depended on the gene and variant spectrum. In phenylketonuria, 87.5% of genotypes and 93.5% (29/31) of alleles (predicted for Europe: 93%) were correctly determined by array analysis. The allele detection rate for cystic fibrosis (75%) was lower because of non-inclusion of relevant variants on the array. In familial hypercholesterolaemia, 101/262 individuals were mutation-positive by sequencing; mutation identification by array was achieved in 68/262 individuals (26%).

Conclusion: Array genotyping is reliable for validated variants and may be used as a cost-effective first-line screening method for common monogenic diseases in a diagnostic setting.

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P16.015.B Clinical utility of the Human Phenotype Ontology terms in the assessment of copy number variants through whole exome sequencing

Barbara Masotto, *Yolanda Moreno-Sáez, Dan Diego-Álvarez, Anna Baquero-Vaquer, Uxía S. Esperón-Moldes, Rosario Ferrer-Avargues, Alfonso Andújar, Tania Otero-Rodríguez, Natalí Riva, Vanesa Felipe-Ponce, Elena Mesa-Rísquez, Isabel Sánchez-Guiu, María D. Ruiz, Roser Martínez-Rubio, Amparo Girós-Pérez, María Sánchez-Ibáñez, Sergio Lois, Oscar Rodríguez, Ángela Arilla-Codoñer, Cristina Torres-Vidal, Norma Aliaga, Laura Cano, Ángela Gaspar, Ana Perpiñán-López, Nuria Serrano, Clara Casañ, Celia Buades-Gomis, Alejandro Romera-López, Cristian M. Moya-Aguilera, Mayte Gil-Borja, Sonia Santillán-Garzón*

Ascires Sistemas Genomicos, Paterna, Valencia, Spain.

The identification of copy number variants (CNVs) through whole exome sequencing (WES) leads to the detection of variants that challenge clinical interpretation. The use of Human Phenotype Ontology (HPO) terms could allow to undermine such challenges. The aim of this study is to assess the clinical utility of HPO terms and determine the coincidence between the suspected diagnosis (when provided) and genetic findings. We selected 30 patients who underwent WES and in whom (likely) pathogenic CNVs were detected. HPO terms were assigned according to the most relevant clinical features in the medical record and each patient was categorized based on the number of terms selected: 1; 2 to 5; 6 to 10; more than 10. HPO terms were compared to those associated with the identified CNV. The suspected diagnosis was compared to the molecular findings. In 26 out of 30 patients an initial diagnosis was presumed; in 14 cases the molecular findings coincided and in 12 they did not. In 4 patients no suspected diagnosis was referred. All patients had at least a single HPO CNV-term match except one. When 2 to 5 terms were selected, the average match was 59.4%, 6 to 10 terms 34.5% and more than 10 terms 20.0%. CNVs identification aided by the selection of HPO terms, highlights the clinical relevance of those variants in which HPO terms match. However, the greater the number of selected terms, the lesser the match. This recalls the importance of an accurate genotype-phenotype correlation after the genetic diagnosis is done.

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P16.016.C Mitochondrial DNA integrity of induced pluripotent stem cells (iPSCs): mandatory screen for unwanted variants before any use of iPSCs

Flavia Palombo[1], *Camille Peron*[2], *Leonardo Caporali*[1], *Angelo Iannielli*[3], *Alessandra Maresca*[1], *Ivano Di Meo*[2], *Claudio Fiorini*[1,4], *Alice Segnali*[2], *Tiina Manninen*[5], *Francesca L. Sciacca*[2], *Ambra Rizzo*[2], *Sonia Levi*[3], *Anu Suomalainen*[5], *Alessandro Prigione*[6], *Vania Broccoli*[3,7], *Valerio Carelli*[1,4], *Valeria Tiranti*[2]

[1]*IRCCS Istituto delle Scienze Neurologiche di Bologna, Bologna, Italy,* [2]*Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy,* [3]*Division of Neuroscience, San Raffaele Scientific Institute, Milano, Italy,* [4]*Department of Biomedical and NeuroMotor Sciences, University of Bologna, Bologna, Italy,* [5]*Research Programs Unit, Molecular Neurology, Biomedicum-Helsinki, University of Helsinki, Helsinki, Finland,* [6]*Max Delbrueck Center for Molecular Medicine (MDC), Berlin, Germany,* [7]*National Research Council (CNR), Institute of Neuroscience, Milano, Italy.*

The generation of inducible pluripotent stem cells (iPSCs) is a revolutionary technique allowing production of pluripotent patient-specific cell lines used for disease modelling, drug screening and cell therapy. Integrity of nuclear DNA (nDNA) is mandatory to allow iPSCs utilization, while quality control of mitochondrial DNA (mtDNA) is rarely included in the iPSCs validation process, although it has been

demonstrated that mtDNA mutations in iPSCs impact on their final phenotype. In this study, we performed mtDNA NGS deep sequencing during the transition from parental fibroblasts to reprogrammed iPSC and to differentiated neuronal precursor cells (NPCs) obtained from controls and patients affected by mitochondrial disorders carrying pathogenic mutations either in mtDNA or nDNA. Our results indicate that at each step, mtDNA variants, including those potentially pathogenic, fluctuate emerging or disappearing. In this way, pathogenic variants reaching a high heteroplasmy load could have a detrimental effect in differentiated cells. Furthermore, the mtDNA haplogroup background might be also relevant for the rate of mtDNA variants emergence in iPSCs. The patients age clearly impacted on the load of somatic variants in parental fibroblasts, but tended to vanish with number of passages in iPSCs. Importantly, we show that also the differentiating step to NPCs may be affected by similar issues. In conclusion, we strongly suggest including mtDNA analysis as an unavoidable assay to obtain fully certified usable iPSCs, confirming the existence of "universal low level heteroplasmy" affecting any human individual. Grant no. 2018-01 to VT and grant RF-2018-12366703 to VC, VB and VT.

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P16.017.D Mosaicism: challenges for next-generation sequencing analysis

Louisa Sanchez[1], *Evelyn Douglas*[1], *David Lawrence*[2], *Julien Soubrier*[1], *Song Gao*[1], *Lesley McGregor*[3], *Christopher Barnett*[3], *Karin Kassahn*[1], *Sui Yu*[1], *Kathryn Friend*[1]

[1]*SA Pathology, North Adelaide, Australia,* [2]*CCB ACRF Cancer Genomics Facility, Adelaide, Australia,* [3]*Women's and Children's Hospital, North Adelaide, Australia.*

It has been estimated that 0.5-8.3% of apparently *de novo* pathogenic variants in autosomal dominant disorders are in fact inherited from a parent with low-level mosaicism for the variant (variant allele frequency (VAF) of 2-29%). This has significant implications for recurrence risk in future pregnancies, and therefore must be given due consideration when analysing next-generation sequencing (NGS) data.

Automated trio analysis pipelines filtering for *de novo* variants may discard these variants as inherited, depending on their parameters. A secondary analysis comparing VAF in the proband and apparently unaffected parents can be used to identify these inherited '*de novo*' variants in clinically relevant genes. Using this approach, we have detected a mosaic *TGFBR2* variant (VAF of 20%) in a clinically unaffected individual with multiple children with Marfan syndrome. It has also lead to the identification of a heterozygous *HECW2* variant in a patient with intellectual disability and severe autistic features. The patient's clinically unaffected father was mosaic for this change (VAF of 30%).

Additional challenges surrounding the identification of mosaicism in NGS data include determining the clinical significance of variants detected with a low VAF in affected probands, and analysis of appropriate tissue types in cases of suspected somatic mosaicism.

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P16.018.A Detection of mtDNA variants from short-read genome sequencing data: analysis of 55 families from a rare disease cohort

Sander Pajusalu[1,2], *Sanna Puusepp*[1,2], *Karit Reinson*[1,2], *Kai Muru*[1,2], *Tiia Reimand*[1,2], *Monica H. Wojcik*[3,4], *Anne O'Donnell-Luria*[3,4], *Katrin Õunap*[1,2]

[1]*Department of Clinical Genetics, Institute of Clinical Medicine, University of Tartu, Tartu, Estonia,* [2]*Department of Clinical Genetics, United Laboratories, Tartu University Hospital, Tartu, Estonia,* [3]*Broad Institute of MIT and Harvard, Broad Center for Mendelian Genomics, Cambridge, MA, USA,* [4]*Division of Genetics and Genomics, Boston Children's Hospital and Harvard Medical School, Boston, MA, USA.*

Introduction: Due to the clinical heterogeneity and nonspecific phenotypes, disorders caused by mtDNA variants cannot be reliably distinguished from the disorders caused by nuclear gene variants. We hypothesized that by analysing mtDNA from genome sequencing data in patients with various severe pediatric-onset disorders, we may increase diagnostic sensitivity.

Materials and Methods: The study group consisted of 55 families with a total of 167 individuals. In all individuals, PCR-free short-read genome sequencing from blood-extracted DNA was previously performed with an average autosomal depth of 37.2x (range 29.2x to 69.2x). After rigorous analysis of nuclear genome variants, 21 families had been diagnosed with either a known or novel monogenic disorders, while the other 34 remained undiagnosed. The mtDNA variants were detected using the GATK4 mitochondrial pipeline. Variants were considered as heteroplasmic if variant allele balance was 5-80%.

Results: The mean mtDNA coverage ranged from 188x to 9,221x with an average of 2,629x. All 61 mother-proband pairs had matching mitochondrial haplogroups, validating the variant calling process. On average one heteroplasmic variant per sample was detected; however, this rate dropped to 0.29 when d-loop regions were excluded. We detected a previously undescribed heteroplasmic missense variant in MT-CYB gene in a patient without a previous molecular diagnosis. Notably, this patient has mitochondrial disease criteria score of 6 indicating probable mitochondrial disorder.

Conclusions: mtDNA variant detection is reliable, quick and cost-effective complementary analysis that can be performed on rare-disease cohorts with available genome sequencing data.

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P16.019.B Multilocus Imprinting Disturbance (MLID) testing in imprinting disorders: Joint position statement to standardize diagnostic testing for MLID

Thomas Eggermann[1], *Deborah Mackay*[2], *Karen Temple*[2], *David Monk*[3], *Eamonn Maher*[4], *Andrea Riccio*[5], *Frederic Brioude*[6], *Agnes Linglart*[6], *Matthias Begemann*[1], *Katja Eggermann*[1], *Miriam Elbracht*[1], *Karen Gronskov*[7], *Jet Bliek*[8], *Paola Lombardi*[8], *Guiomar Perez de Nanclares*[9], *Masayo Kagami*[10], *Irene Netchine*[6], *Tsutomu Ogata*[10], *Zeynep Tümer*[7]

[1]Institute of Human Genetics, Aachen, Germany, [2]University of Southampton, Southampton, United Kingdom, [3]University of East Anglia, Norwich, United Kingdom, [4]University of Oxford, Oxford, United Kingdom, [5]University of Naples II, Naples, Italy, [6]Sorbonne University, Paris, France, [7]Kennedy Center, Glostrup, Denmark, [8]University of Amsterdam, Amsterdam, Netherlands, [9]Hospital Universitario Araba. Sede Txagorritxu, Vitoria-Gasteiz, Spain, [10]Hamamatsu University School of Medicine, Hamamatsu, Japan.

With the implementation of comprehensive tests in the genetic diagnosis of imprinting disorders (ImpDis), there is a substantial increase in observations of novel, complex and heterogeneous molecular findings. Notably, a subset of patients with Beckwith-Wiedemann and Silver-Russell syndromes, as well as transient neonatal diabetes mellitus, the 14q32 and GNAS associated ImpDis, show multilocus imprinting disturbances (MLID), i.e. aberrant methylation at multiple imprinted loci in the genome. As recent studies show, MLID contributes to the clinical heterogeneity of ImpDis, and an increased risk for reproductive failure in the families. In fact, the precise identification of the type of defect is often a prerequisite for the clinical management and genetic counselling in families with ImpDis. The first cases of MLID and its clinical relevance were reported more than 15 years ago, and reliable commercial laboratory tests are available; despite this, there is no implementation of MLID testing in routine diagnostics, nor any consensus on the definition of MLID, clinical indications for MLID testing, or molecular indications of imprinted loci to be tested. Based on their long experience with molecular and clinical diagnostics of imprinting disorders, genetic counselling and treatment, the authors will address these open questions, and suggest the conditions of implementation of MLID testing in routine diagnostics.

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P16.020.C The clinical validation of GeneProof PCR Kits for detection of thrombotic polymorphisms on myCROBE® Fully Automated Instrument

Kamila Poláková[1], *Petr Janda*[1], *Dita Bednářová*[1], *Lenka Tomáiková*[2], *Ivan Slí*[2], *Eva Jansová*[1,3]

[1]GeneProof a. s., Brno, Czech Republic, [2]Unilabs Slovensko, s. r. o., Bánská Bystrica, Slovakia, [3]Mendel University in Brno, Department of Chemistry and Biochemistry, Brno, Czech Republic.

Introduction: Combination of acquired and inherited risk factors can lead to disbalance of coagulation system which is related to many serious disorders. Each assay needs to be detected separately and without automatization, it is a time-consuming process. The aim of this study was to evaluate clinical performance characteristics of the innovated GeneProof PCR assays intended for diagnostics of thrombophilic mutations in myCROBE® Fully Automated Instrument.

Materials and Methods: The clinical validation was performed on 842 whole blood samples in total, 184 for Factor II, 186 for Factor V, 111 for Factor XIII, 123 for MTHFR A1298C, 123 for MTHFR C677T and 115 for PAI-1. The samples were extracted, detected, and evaluated in myCROBE® Fully Automated Instrument using innovated GeneProof PCR Kits for myCROBE. The correct genotype was confirmed in collaboration with clinical site at the Unilabs Slovensko, s. r. o. using CE IVD GeneProof PCR Kits with croBEE 201A NA Extraction Kit.

Results: Comparison of myCROBE PCR Kits and GeneProof CE IVD PCR Kits demonstrated high reliability which is expressed by Negative percentage agreement (FII, FV, FXIII, PAI-1 - 100 %, MTHFR A1298C - 98.04 %, MTHFR C677T - 96.72 %) and Positive percentage agreement (FII, FV, FXIII, MTHFR A1298C, PAI-1 - 100 %, MTHFR C677T - 96.77 %).

Conclusions: The results of clinical validation demonstrate a very good diagnostic parameter of the GeneProof assays and together with myCROBE® Fully Automated Instrument proved to be very convenient and time-saving tool for testing of thrombophilic mutations in clinical routine.

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P16.021.D Virtual genetic assessments for neonatal intensive care unit patients

Demetra Georgiou[1,2], *Libuse Pazderova*[1], *Harry Leitch*[2], *Emanuel Curetean*[1], *Teena Ferguson*[1], *Jan Cobben*[2]

[1]Imperial College Healthcare NHS Trust, London, United Kingdom, [2]North West Thames Regional Genetics Service, London, United Kingdom.

Introduction: The North West Thames Regional Genetics Service, supports a number of a highly specialised Neonatal units, which are geographically distant. The introduction of the NHS Rapid exome sequencing for acutely ill neonates and children (RAPS) in 2020, increased the need for urgent ward consultations. The COVID19 pandemic, has made the delivery of urgent Genetic ward consultations extremely challenging.

Methods: A digital solution pilot has been developed to address this challenge which started with videoconferencing. We since acquired a mobile trolley with a high definition camera that is placed in the busiest neonatal ward in our region. We utilised NHS approved IT solutions to facilitate quicker assessment with virtual remote consultations, utilising streaming video to phenotype the child and consult with parents and Neonatologists. **Outcomes:** Clinical geneticists can remotely review neonates and provide support promptly and efficiently. With the use of remote video calls for the assessment of acutely unwell neonates in North West London, the diagnostic rate of RAPS DNA analysis appeared to be very high (~70%). This indicates that virtual remote phenotyping to guide the DNA analysis, can be successfully used instead of live ward consultations. We will report on quality and speed of assessment, as well as clinician and patient satisfaction. We will furthermore provide a financial evaluation of this novel service.

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P16.022.A Importance of careful transcript identification for pathogenicity assessment of the rare and confusing variant in the SCN5A gene

Anna Shestak[1], *Leonid Makarov*[2], *Vera Komolyatova*[2], *Liubov Skorodumova*[3], *Irina Kolesnikova*[3], *Eduard Generozov*[3], *Elena Zaklyazminskaya*[1]

[1]Petrovsky National Research Center of Surgery, Moscow, Russian Federation, [2]Center for Syncope and Cardiac Arrhythmias in Children and Adolescents of the Federal Medical Biological Agency, Moscow, Russian Federation, [3]Federal Research and Clinical Center of Physical-

Chemical Medicine of Federal Medical Biological Agency, Moscow, Russian Federation.

Introduction: Continuously growing request for Next Generation Sequencing (NGS) drive the automatization of processes from primer design to variant calling and interpretation. The purpose of this study is to demonstrate the rare situation when the variant was correctly evaluated only by combination of NGS techniques and Sanger sequencing.

Methods: Female sportsman (15 y.o.) underwent genetic counseling and DNA testing due to QT interval prolongation registered during ECG Holter monitoring. Genetic testing of the proband was performed in two independent laboratories. Primary DNA testing was performed by WES using the Ion Proton™ System. Target panel sequencing of 11 genes encoding cardiac ion channels was performed using PGM IonTorrent. Search for a variants in noncanonical and canonical exons 6 was performed by Sanger sequencing.

Results: The cascade familial screening and control re-sequencing were provided for proband with identified genetic variant p.S216L (g.38655290G>A, NM_198056.2:c.647C>T, rs41276525) in the canonical exon 6 of the *SCN5A* gene after receiving data from another laboratory. Control Sanger and NGS sequencing revealed the absence p.S216L in the canonical exon 6 and confirmed the presence of p.S216L (g.38655522G>A, c.647C>T, rs201002736) in the noncanonical exon 6 of the *SCN5A* gene. The identified variant was re-interpreted. The noncanonical transcripts of the exon 6 of the *SCN5A* gene is poorly represented in cardiac tissue (gnomAD). The detected variant was found in proband's healthy mother.

Conclusion: The correct interpretation of genetic data requires close cooperation between cliniciants and researchers. It can help to avoid financial costs and stress for probands and families.

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P16.025.D Circulating exosomal miRNAs as potential biomarkers of Parkinson's disease

Selene De Benedittis[1], *Ida Manna*[2], *Andrea Quattrone*[1], *Enrico Iaccino*[3], *Gennarina Arabia*[1], *Aldo Quattrone*[2,4]

[1]*Institute of Neurology, Department of Medical and Surgical Sciences, Magna Graecia University, Catanzaro, Italy, Catanzaro, Italy,* [2]*Institute of Molecular Bioimaging and Physiology (IBFM), National Research Council (CNR), Section of Germaneto, 88100 Catanzaro, Italy, Catanzaro, Italy,* [3]*Department of Experimental and Clinical Medicine, University "Magna Graecia" of Catanzaro, 88100 Catanzaro, Italy, Catanzaro, Italy,* [4]*Neuroscience Research Center, University Magna Graecia, 88100 Catanzaro, Italy, Catanzaro, Italy.*

Introduction: Parkinson's disease (PD) is a progressive neurodegenerative disorder with the clinical characteristics of bradykinesia, tremor and rigidity. MicroRNAs (miRNAs) are small non-coding RNAs observed in biological fluids, where can be shuttled in exosome, extracellular vesicles that play an important role in cell-to-cell communication. miRNAs have been investigated and suggested as important biomarkers for the evaluation of disease, including neurodegenerative diseases. Therefore, we selected a set of miRNAs commonly studied in neurodegenerative diseases, and studied their expression levels in serum exosomes to evaluate their feasibility and potential application as biomarkers in PD.

Materials and Methods: 24 patients with PD were included in study group, and 24 age- and sex-matched healthy subject were included in control group. The blood samples were processed for serum isolation, and exosomes isolation and miRNAs extraction were done using commercial kits. The expression of exosomal miRNAs was analyzed by reverse transcriptase quantitative Real-time PCR (RT-qPCR). The relative expressions of miRNAs were normalized to the exogenous reference cel-miR-39 expression. miRNAs data analysis was performed using the $2-\Delta Ct$ method. Differences between groups were assessed using Student's t-test and results were considered to have statistically significant difference if $p < 0.05$.

Results: Among all tested miRNAs only 2 were differentially expressed. Compared with controls, patients with PD showed a significantly higher expression of circulating exosomal miR-150-5p, while exosomal miR-132-3p was significantly lower.

Conclusions: The development of biomarkers based on miRNAs requires further study, but these preliminary results suggested that exosomal miR-150-5p and miR-132-3p might be candidate PD diagnostic biomarkers.

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P16.026.A The collection of samples from pregnant women at different stages of gestation to search for early biomarkers of pregnancy complications

Roman A. Illarionov[1,2,3], *Olga V. Pachuliia*[1], *Nataliya O. Yurkina*[1], *Maria G. Butenko*[1], *Elena S. Vashukova*[1], *Andrey S. Glotov*[1]

[1]*D.O. Ott The Research Institute of Obstetrics, Gynecology and Reproductology, Saint-Petersburg, Russian Federation,* [2]*Saint-Petersburg State Institute of Technology (Technical University), Saint-Petersburg, Russian Federation,* [3]*Saint-Petersburg University, Saint-Petersburg, Russian Federation.*

Introduction: Biobanks today represent a modern basic platform providing access to quality biomaterial. The search for early biomarkers of pregnancy complications will reveal new perspectives in early diagnosis. The aim of the project is to create a collection of samples of pregnant women at different stages of gestation to search for early biomarkers of pregnancy complications.

Material and methods: Collection, storage and processing of biological samples of pregnant women at different stages of gestation and associated clinical data, quality control.

Results. The collection includes peripheral blood samples from 204 women; whole blood, serum, plasma, buffy coat, and urine were collected during pregnancy, and placenta and umbilical cord blood samples were collected during labor. A clinical and anamnestic information was obtained about each pregnant woman, which includes data on the woman's health status, the course of gestation, and the outcome of pregnancy. The quality control of the collection samples was carried out. The study of the microRNA expression profile in healthy women at different stages of gestation and different types of biomaterial was carried out.

Conclusion: The creation of a collection of samples of pregnant women is a significant groundwork for future fundamental and applied research in various fields of biomedicine. Research on the basis of the collection may allow a study of the pathogenetic mechanisms of various gestational complications, and the development of new methods for their diagnosis and treatment. Funding. This study was financially supported by a Russian Science Foundation grant 19-75-20033.

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P16.027.B Diagnostic Power and Clinical Impact of Exome Sequencing in a Cohort of 500 Patients with Rare Diseases

Caio Robledo D. A. C. Quaio[1], *Caroline Monaco Moreira*[1], *Gil Monteiro Novo Filho*[1], *Patricia Rossi Sacramento-Bobotis*[1], *Sandro Felix Perazzio*[1], *Aurelio Pimenta Dutra*[1], *Michele Groenner Penna*[1], *Rafael Alves da Silva*[1], *Rodrigo Fernandes Ramalho*[1], *Rafaela Rogerio Floriano de Sousa*[1], *Miguel Mitne-Neto*[1], *Wagner Antonio da Rosa Baratela*[1], *Chong Ae Kim*[2]

[1]*Fleury Medicina e Saúde, Sao Paulo - SP, Brazil,* [2]*Instituto da Criança, Hospital das Clínicas HCFMUSP, Faculdade de Medicina, Universidade de São Paulo, Sao Paulo - SP, Brazil.*

Rare diseases comprise a diverse group of conditions, most of which involve genetic causes. We describe the variable spectrum of findings and clinical impacts of exome sequencing (ES) in a cohort of 500 patients with rare diseases. In total, 164 primary findings were reported in 158 patients, representing an overall diagnostic yield of 31.6%. Most of the findings (61.6%) corresponded to autosomal dominant conditions,followed by autosomal recessive (25.6%) and X-linked (12.8%) conditions. These patients harbored 195 variants, among which 43.6% are novel in the literature. The rate of molecular diagnosis was considerably higher for prenatal samples (67%; 4/6), younger children (44%; 24/55), consanguinity (50%; 3/6), gastrointestinal/liver disease(44%; 16/36) and syndromic/malformative conditions (41%; 72/175). For 15.6% of the cohort patients, we observed a direct potential for the redirection of care with targeted therapy, tumor screening, medication adjustment and monitoring for disease-specific complications. Secondary findings were reported in 37 patients (7.4%). Based on cost-effectiveness studies in the literature, we speculate that the reports of secondary findings may influence an increase of 123.2 years in the life expectancy for our cohort, or 0.246 years/cohort patient. ES is a powerful method to identify the molecular bases of monogenic disorders and redirect clinical care.

C.D.A.C. Quaio: A. Employment (full or part-time); Significant; Fleury Medicina e Saude. **C. Moreira:** A. Employment (full or part-time); Significant; Fleury Medicina e Saude. **G. Novo Filho:** A. Employment (full or part-time); Significant; Fleury Medicina e Saude. **P. Sacramento-Bobotis:** A. Employment (full or part-time); Significant; Fleury Medicina e Saude. **S. Perazzio:** A. Employment (full or part-time); Significant; Fleury Medicina e Saude. **A. Dutra:** A. Employment (full or part-time); Significant; Fleury Medicina e Saude. **M. Groenner Penna:** A. Employment (full or part-time); Significant; Fleury Medicina e Saude. **R. Silva:** A. Employment (full or part-time); Significant; Fleury Medicina e Saude. **R. Ramalho:** A. Employment (full or part-time); Significant; Fleury Medicina e Saude. **R. Sousa:** A. Employment (full or part-time); Significant; Fleury Medicina e Saude. **M. Mitne-Neto:** A. Employment (full or part-time); Significant; Fleury Medicina e Saude. **W. Baratela:** A. Employment (full or part-time); Significant; Fleury Medicina e Saude. **C. Kim:** None.

P16.028.C Laboratory proactivity can solve non-diagnostic whole exome sequencing: Probing new genes in old data

Juliana Heather Vedovato-dos-Santos[1], *João P. Kitajima*[1], *Juliana Jordão*[1], *Erika L. Freitas*[1], *Mara D. O. Ribeiro*[1], *Julia G. Salomão*[1], *Luiza L. P. Ramos*[1], *Larissa A. Costa*[1], *Luciana Bispo*[1], *Fernanda Monti*[1,2], *Caroline Freitas*[1], *Fabíola Paoli Monteiro*[1], *Fernando Kok*[1,3]

[1]*Mendelics Genomic Analysis, São Paulo, Brazil,* [2]*Jô Clemente Institute, São Paulo, Brazil,* [3]*University of São Paulo, School of Medicine, São Paulo, Brazil.*

Introduction: Whole exome sequencing (WES) is often the most efficient and most widely available diagnostic tool for patients under investigation for a Mendelian disorder, however, a significant proportion of clinical exomes (60%-75%) is non-diagnostic. Reanalysis is often the next step in the diagnostic odyssey of many patients. We propose a more efficient approach to the reanalysis of WES data, based on our experience applying this "targeted-reanalysis" method to 299 patients under investigation for genetic disorders, screened over 6 years (2015-2021).

Materials and Methods: Our approach to reanalysis consists of three steps: 1. Periodically searching the medical literature for new gene-disease associations; 2. Actively searching our database for patients with variants in these genes; 3. Performing a targeted reanalysis of those variants, considering only patients whose original WES data was non-diagnostic.

Results: The diagnostic yield of this targeted reanalysis was 24.4%, with a definite diagnosis identified for 73 of the 299 patients. In 7 additional cases, we reported variants of unknown significance relevant to the patient's phenotype, which we strongly believe are responsible for the clinical features.

Conclusions: Reanalysis of non-diagnostic WES data is a proven method of maximizing the diagnostic yield of whole exome sequencing in a clinical setting. It is usually initiated by clinical genetics providers, and the diagnostic yield is generally between 10 and 12%. Our data demonstrate that targeted reanalysis significantly increases the diagnostic yield, further highlighting the potential of this method for solving previously undiagnosed cases.

J.H. Vedovato-dos-Santos: A. Employment (full or part-time); Significant; Mendelics Genomic Analysis. **J.P. Kitajima:** A. Employment (full or part-time); Significant; Mendelics Genomic Analysis. **J. Jordão:** A. Employment (full or part-time); Significant; Mendelics Genomic Analysis. **E.L. Freitas:** A. Employment (full or part-time); Significant; Mendelics Genomic Analysis. **M.D.O. Ribeiro:** A. Employment (full or part-time); Significant; Mendelics Genomic Analysis. **J.G. Salomão:** A. Employment (full or part-time); Significant; Mendelics Genomic Analysis. **L.L.P. Ramos:** A. Employment (full or part-time); Significant; Mendelics Genomic Analysis. **L. A. Costa:** A. Employment (full or part-time); Significant; Mendelics Genomic Analysis. **L. Bispo:** A. Employment (full or part-time); Significant; Mendelics Genomic Analysis. **F. Monti:** A. Employment (full or part-time); Significant; Mendelics Genomic Analysis. **C. Freitas:** A. Employment (full or part-time); Significant; Mendelics Genomic Analysis. **F.P. Monteiro:** A. Employment (full or part-time); Significant; Mendelics Genomic Analysis. **F. Kok:** A. Employment (full or part-time); Significant; Mendelics Genomic Analysis.

P16.029.D Detecting inversions in routine molecular diagnosis

Edwige Kasper[1], *Sophie Coutant*[1], *Sandrine Manase*[1], *Stéphanie Vasseur*[1], *Pierre Macquère*[1], *Gaëlle Bougeard*[1], *Laurence Faivre*[2], *Olivier Ingster*[3], *Thierry Frebourg*[1], *Stéphanie Baert-Desurmont*[1], ***Claude Houdayer***[1]

[1]*Normandie Univ, UNIROUEN, Inserm U1245, CHU Rouen, Department of Genetics and reference center for developmental disorders, FHU G4 Génomique, F-76000 Rouen, France, Rouen, France,* [2]*Centre de Lutte Contre le Cancer Georges François Leclerc, Dijon, France32Centre de Génétique, Hôpital d'Enfants, CHU Dijon, Dijon, France, Dijon, France,* [3]*Department of Genetics, University Hospital Centre Angers, Angers, Pays de la Loire, France, Angers, France.*

Inversions, i.e. a change in orientation of a segment of DNA, are a recognized cause of human diseases. Despite considerable progress in sequencing and bioinformatics approaches, they remain often

overlooked due to their balanced nature and mapping issues. Inversions can have brutal effects by disrupting the coding sequence or more subtle impacts on gene expression. We described two families that exemplify these aspects.The first family (F1) displayed a sibship with 2 constitutional mismatch repair deficiency (CMMRD) patients and a family history of colon cancer in the maternal branch only. The second family (F2) displayed 2 sisters affected by endometrial cancer and one brother with colon cancer. Both parents were deceased and no information was available.

The families were analyzed using an "augmented" panel including the major colon cancer genes with their intronic and flanking genomic regions. Inversions were called using GRIDSS. In F1, we evidenced a PMS2 pathogenic splice variation and, in the paternal branch, a low penetrance PMS2 inversion encompassing the promoter region to intron 1, thereby confirming the CMMRD diagnosis. In F2, we found an inversion involving the 5′ part of MSH6 and the 3′ part of the nearby gene ANXA4, explaining the familial history.Inversion detection mandates "augmented" panels or WGS and dedicated tools, but makes a valuable contribution to the diagnostic rate. Moreover, one can expect that a fraction of inversions, which may alter slightly gene expression, would act also as low-risk or modifying genetic factors.

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P16.030.A Translational diagnostics program: an in-house pipeline to validate genetic variants in children with undiagnosed and rare diseases

Jordi Pijuan[1], *María Rodríguez-Sanz*[1], *Daniel Natera-de Benito*[2], *Anna Altisent*[1], *Carlos Ortez*[2,3], *Raúl Benítez*[4], *Andrés Nascimento*[2,3], *Janet Hoenicka*[1,3], *Francesc Palau*[1,3,5]

[1]*Laboratory of Neurogenetics and Molecular Medicine – IPER, Institut de Recerca Sant Joan de Déu, Barcelona, Spain,* [2]*Neuromuscular Unit, Department of Pediatric Neurology, Hospital Sant Joan de Déu, Barcelona, Spain,* [3]*Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Barcelona, Spain,* [4]*Automatic Control Department and Biomedical Engineering Research Center, Universitat Politècnica de Catalunya, Barcelona, Spain,* [5]*Department of Genetic Medicine – IPER, Hospital Sant Joan de Déu and Hospital Clínic University of Barcelona, Barcelona, Spain.*

Introduction: There are approximately 7,000 rare diseases affecting 350 million people worldwide. Diagnosis is essential for the management and treatment of patients. Next-generation sequencing have hugely improved the prospects of obtaining a molecular diagnosis, but genetic variants of uncertain significance (VUS) or inconsistent patient phenotype prevent the diagnosis. Herein, we show the results of the in-house Translational Diagnostics Program (TDP) to validate genetic variants as part of the diagnostic process with the close intramural collaboration between physicians, clinical scientists, and researchers.

Materials and methods: 43 undiagnosed patients in which the exome test showed a VUS or a phenotype-genotype incongruity were selected. The TDP pipeline comprises four steps: (i) precision phenotype evaluation, (ii) literature review and *in silico* biology studies on the pathogenicity of variants (iii) experimental functional studies (e.g., protein subcellular localization, protein interactions and activity) of transfected cells lines or patients' fibroblasts, and (iv) diagnosis decision-making.

Results: Reassessment of the phenotype and *in silico* biology experiments allowed diagnosis in 10 patients. In the remaining 33 patients, we performed comparative computational analysis of confocal microscopy images and experiments related to protein function. Biological validation was achieved in 14 patients, the genetic variant was rejected in two patients, and 17 cases are ongoing. The overall diagnostic rate is currently 55.8%.

Conclusions: PDT supports and resolves intramural medical problems when the clinical significance of the patient's variant is unknown or clinically inconsistent.

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P16.031.B Transthyretin, Retinol-binding protein, retinol and T4 circulating levels in the Mallorca population of TTR V30M carriers

Eugenia Cisneros-Barroso, *Juan González, Adrián Rodríguez, Tomás Ripoll-Vera, Inés Losada*

Fundació Institut d'Investigació Sanitària Illes Balears – IdISBa, Spain, Spain.

To date, more than 140 mutations in the TTR gene have been described. Val30Met (p.Val50Met) is the most common one in general and in particular in the island of Majorca (Spain) where ATTRV30M amyloidosis is considered endemic. Serum Transthyretin levels have already been reported to be decreased for Portuguese, Japanese and Swedish TTR V30M carriers. However, no studies have been done in the population of Mallorca. We have been able to identify 200 TTR V30M from the endemic foci of Mallorca. Data on serum TTR levels are available for 100 of them. We then show the analysis of serum transthyretin levels in the TTRV30M carriers from the endemic foci of Mallorca. TTR circulates primarily as a 55 KDa tetramer. In the blood, the TTR tetramer binds to the retinol binding protein complex and a small amount of T4 and transports it to different tissues. Although TTR is the only known RBP transporter that facilitates the transport of retinol, there are two other T4 transporter proteins: albumin and thyroxine binding globulin. TTR plays a secondary role in the transport of T4 in blood while, although, it is the main transporter at the level of cerebrospinal fluid. Then we have also determined the levels of retinol, RBP and T4 in 100 TTRV30M carriers from Mallorca. This is the first study that focuses on the study of TTR and its circulating partners blood levels in the endemic foci of Mallorca island.

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P16.033.D Solving the unsolved: 4 years of experience of the Italian Telethon Undiagnosed Diseases Program

Raffaele Castello[1], *Annalaura Torella*[1,2], *Manuela Morleo*[1], *Michele Pinelli*[1], *Francesco Musacchia*[1], *Alessandra Varavallo*[1,2], *Margherita Mutarelli*[1,3], *Sandro Banfi*[1,2], *Angelo Selicorni*[4], *Milena Mariani*[4], *Silvia Maitz*[5], *Valeria Capra*[6], *Andrea Accogli*[6], *Marcello Scala*[6], *Vincenzo Leuzzi*[7], *Anna Commone*[7], *Francesca Nardecchia*[7], *Serena Galosi*[7], *Mario Mastrangelo*[7], *Donatella Milani*[8], *Corrado Romano*[9], *Pinella Failla*[9], *Donatella Greco*[9], *Chiara Pantaleoni*[10], *Claudia Ciaccio*[10], *D'Arrigo Stefano*[10], *Nicola Brunetti-Pierri*[1,11], *Giancarlo Parenti*[1,11], *Gerarda Cappuccio*[1,11], *Andrea Ballabio*[1,11], *Antonietta Coppola*[12], *Martino Montomoli*[13], *Maria Alice Donati*[14], *Teresa Mattina*[15],

Marcella Zollino[16], Simona Amenta[16], Albina Tummolo[17], Claudia Santoro[18], Giulio Piluso[2], Valerio Bonolis[2], Roberta Zeuli[2], Giancarlo Blasio[2], Daniele De Brasi[19], Francesca Del Vecchio Blanco[2], Angela Peron[20], Gennaro Oliva[21], Diego di Bernardo[1,22], Ermanno Rizzi[23], Giorgio Casari[24,1], Vincenzo Nigro[1,2]

[1]Telethon Institute of Genetics and Medicine, Pozzuoli, Naples, Italy, [2]Department of Precision Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy, [3]Institute of Applied Sciences and Intelligent Systems "Eduardo Caianiello", ISASI, National Research Council, Naples, Italy, Pozzuoli, Naples, Italy, [4]Department of Pediatrics, ASST Lariana Sant'Anna Hospital, San Fermo della Battaglia, Italy, [5]MBBM Foundation, Monza, Italy, [6]Neuroscience Department, Giannina Gaslini Institute, Genoa, Italy, [7]Department of Human Neuroscience, Sapienza University of Rome, Rome, Italy, [8]Pediatric Highly Intensive Care Unit, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy, [9]Oasi Research Institute - IRCCS, Troina, Italy, [10]Developmental Neurology Unit, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy, [11]Department of Translational Medicine, Section of Pediatrics, Federico II University, Naples, Italy, [12]Department of Neuroscience and Reproductive and Odontostomatological Sciences, Federico II University, Naples, Italy, [13]Pediatric Neurology, Neurogenetics and Neurobiology Unit and Laboratories, Neuroscience Department, A Meyer Children's Hospital, University of Florence, Florence, Italy, [14]Metabolic and Muscular Unit, Regional Reference Centre Expanded Newborn Screening, Meyer Children Hospital, Florence, Italy, [15]Department of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy, [16]Institute of Genomic Medicine, Catholic University, Gemelli Hospital Foundation, Rome, Italy, [17]Department of Metabolic Diseases, Clinical Genetics and Diabetology, Giovanni XXIII Children's Hospital, Bari, Italy, [18]Pediatric Surgery, Department of Women, Children, General, and Specialist Surgery, Campania University "Luigi Vanvitelli", Naples, Italy, [19]Department of Pediatrics, AORN Santobono Pausilipon, Naples, Italy, [20]Child Neuropsychiatry Unit-Epilepsy Center (Service of Medical Genetics), San Paolo Hospital, Department of Health Sciences, Università degli Studi di Milano, Milan, Milan, Italy, [21]Institute for High Performance Computing and Networking, National Research Council, Naples, Italy, [22]Department of Chemical, Materials and Industrial Production Engineering, University of Naples Federico II, Naples, Italy, [23]Fondazione Telethon, Milan, Italy, [24]Vita-Salute San Raffaele University, Milan, Italy.

Rare genetic diseases affect millions of children worldwide. Diagnosis is essential to carry out scientific research on the causes and new treatments, but it is unavailable for many cases. The Telethon Undiagnosed Diseases Program (TUDP) is a multicentric Italian national program with the objective of studying a broad spectrum of rare paediatric-onset monogenic disorders, characterized by severe multisystem manifestations, neurological involvement, and dysmorphic features. The TUDP has adopted whole exome sequencing (WES) of the child and its parents as entry-level test. After phenotypic annotation and trio/quartet WES (completed in 573 families), the TUDP network found a causative mutation in 49% of cases. In particular, in 79% of diagnosed patients a phenotypic extension in known disease genes was identified, while for the remaining 21% variants in newly discovered disease genes were identified. A significative proportion of TUDP patients have putative pathogenic mutations in genes not fully characterized /not associated with Mendelian diseases. Assuming all experimental steps are performed according to a high standard, the very high success rate of TUDP could be the effect of the rigorous patient selection criteria used. Another explanation for the success rate of TUDP refers to the higher parental age in Italy, which is associated with the easier identification of *de novo* dominant exonic mutations, present in 68% of cases solved by TUDP. In addition, TUDP benefited from a close well-orchestrated collaboration between bioinformaticians, more than 50 clinical geneticists and basic scientists, and from the interactions between academia, 14 Italian pediatric hubs, and patient families.

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P16.034.A Genomic analysis; gene panel versus phenotype based variant filtering, a comparative analysis

Cleo C. van Diemen, Kristin M. Abbott, Annemieke van der Hout, Inge Mulder, Kim de Lange, Krista van Dijk-Bos, Lennart F. Johansson, Joeri K. van der Velde, Dennis Hendriksen, Bart Charbon, S van den Roek, Martine T. Meems-Veldhuis, Henny H. Lemmink, Morris A. Swertz, Birgit Sikkema-Raddatz, ***Marielle E. van Gijn***

University Medical Center Groningen, Groningen, Netherlands.

Gene panel and whole exome sequencing is now mainstream in clinical practice. Despite many different software tools to support analysis, the diagnostic yield is still limited to 20-70%, depending on the phenotype. Moreover, the process remains time consuming and labor intensive. Currently, the most common data-analysis strategy is the use of fixed gene-panels. However, flexible, phenotype-based analysis is gaining attention and might be preferable for complex phenotypes or heterogeneous diseases. To determine which strategy is most efficient and results in the highest diagnostic yield, we compared three different analysis strategies: firstly, our current diagnostically used Alissa software (Agilent), a gene-panel based analysis; secondly Moon software (Diploid), a phenotype-based analysis using artificial intelligence; thirdly an in-house developed variant interpretation pipeline (VIP), using both strategies. We prospectively included 300 patients (either in trio design or singletons), a diverse group reflecting our diagnostic spectrum. The reasons for referral were developmental delay, fetus with multiple congenital abnormalities at ultrasound, critically ill children from intensive care unit, cardiomyopathy, hereditary cancer, epilepsy, and skin abnormalities. The outcome measures were number of diagnoses, estimated time needed for analysis, and number of variants left requiring manual interpretation. Preliminary results indicate that the time needed for analysis and amount of variants left for interpretation was the lowest in the phenotype-based analysis. The number of diagnoses in the epilepsy group was higher, however, in the gene-panel based analysis. Potential additional diagnoses are currently being investigated. The preliminary data indicate that it depends on the phenotype of the patient which strategy is preferable.

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P16.035.B Whole Exome Sequencing in critically ill neonates and infants: diagnostic yield and predictability of monogenic diagnoses

Tasja Scholz[1], *Tatjana Bierhals*[1], *Fanny Kortüm*[1], *Davor Lessel*[1], *Christian Kubisch*[1], *Dominique Singer*[2], *Martin Ernst Blohm*[2], *Anna Perez*[2], *Jessika Johannsen*[3], *Jonas Denecke*[3], *René Santer*[3], *Philipp Deindl*[2], *Maja Hempel*[1]

[1]*Institute of Human Genetics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany,* [2]*Division of Neonatology and Pediatric Intensive Care, Department of Pediatrics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany,* [3]*Department of Pediatrics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany.*

Introduction: Monogenic diseases play an important role in critically ill neonates and infants treated in the intensive care unit (ICU). This study aims to determine the diagnostic yield of whole-exome sequencing (WES) for monogenic diseases and to identify phenotypes associated with a genetic etiology.

Methods: From March 2017 to March 2020, a comprehensive diagnostic work-up including WES was performed in a single academic center in 61 unrelated, critically ill neonates and infants below 1 year of age with an unknown underlying disease. We conducted 59 Trio-WES, 1 Duo-WES, and 1 Single-WES analyses. Symptoms were classified according to the Human Phenotype Ontology (HPO).

Results: The overall molecular genetic diagnostic rate within was 46% (28/61), with a genetic diagnosis of 50% (15/30) in preterm neonates. Identifying the genetic cause of disease has facilitated an individualized management in the majority of patients. A positive or negative predictive power of specific clinical features for a genetic diagnosis could not be observed.

Conclusion: WES is a powerful non-invasive diagnostic tool in critically ill neonates and infants with a high diagnostic rate. We recommend initiating WES as early as possible due to the impact on management and family counseling. Recommendations regarding the clinical utility of WES in critically ill neonates and infants should not be based on phenotype alone. We present a clinical workflow for the application of WES for critically ill neonates and infants in an interdisciplinary setting.

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P16.036.C Identification of structural variation in constitutional disorders by optical genome mapping

Andy Wing Chun Pang, *Alex Hastie, Alka Chaubey*

Bionano Genomics, San Diego, CA, USA.

Chromosome disorders form a major category of genetic disease. Karyotype analysis, fluorescence in situ hybridization (FISH) and microarrays are currently used in clinical cytogenetics and molecular diagnostics. CMA is the first line test recommended by American College of Medical Genetics (ACMG), American Academy of Pediatrics (AAP), and America Academy of Neurology (AAN), however, CMA miss many classes of structural variants such as balanced translocations and inversions and cannot make calls in in segmental duplications and repeats. Recent advances in optical genome mapping (OGM) have the potential to address these shortcomings. Bionano Genomics' Saphyr platform extracts megabases long DNA, labels at specific motifs, and linearizes through NanoChannels. These molecules at least 150kbp in length are then digitized and assembled *de novo* into chromosomal-arm length optical maps. Due to the long length of the assembled maps, large structural variants (SVs) can be captured by aligning to the public human reference assembly. We applied this platform to study the genomes of multiple constitutional disorders. We detected segmental duplication mediated deletions such as a 1.9 Mbp deletion in 7q11.23 (Williams-Beuren Syndrome), and a 3.7 Mbp deletion in 17p11.2 in (Smith-Magenis Syndrome). Optical mapping also captured 12q+, trisomies 13 and 21, and it detected rearrangements such as t(2;10) and t(9;21) translocations. Furthermore, OGM detected repeat expansions in Fragile X and repeat contractions in FSHD. In conclusion, in one platform, optical genome mapping has the potential to identify a broad range of genomic abnormalities, and to improve the characterization of SVs.

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P17 Bioinformatics, Machine Learning and Statistical Methods

P17.002.A Bioinformatic approach to detect alternative splicing and integration of omics data for the study of neurological diseases

Valérie Triassi[1,2], *Éric Bareke*[1], *Sébastien Audet*[1,3], *Marjorie Labrecque*[1,2], *Martine Tétreault*[1,3]

[1]*CHUM Research Center, Montréal, QC, Canada,* [2]*Bioinformatics program, Department of biochemistry and molecular medicine, Université de Montréal, Montréal, QC, Canada,* [3]*Department of Neuroscience, Université de Montréal, Montréal, QC, Canada.*

Interpretation of genetic data is a big challenge and genetic testing often results in the identification of variants of unknown significance. Several of these variants may disrupt normal RNA splicing or affect gene expression. The aim of this project is to set up a pipeline in order to identify and characterise variants of interest in a pathological setting. To determine if variants have a functional impact, our pipeline focuses on alternative splicing as well as the integration of exome and transcriptome data. We used SpliceAI, an efficient tool for predicting the impact of variants on alternative splicing and rMATS, which best predicts if an alternative splicing event occurs from exon-intron junctions. A combination of these two tools further increases detection efficiency. Then, we integrated DNA-seq and RNA-seq variants to detect allelic imbalances. Custom scripts also integrate variants with the list of alternative splicing events, previously generated using SpliceAI and rMATS in order to associate a variant to a splicing junction for both data sets (DNA and RNA). Gene carrying variants or alternative splicing events were also assessed differential expression. The variants sought are rare and therefore require a consistent and efficient pipeline in order to facilitate the detection of these events. Moreover, the integration of omics data makes it possible to obtain a research track on genes which are more interesting and to be prioritized. This pipeline makes it possible to optimize the search for potentially pathogenic variants in heterogeneous neurological diseases, such as myopathies and muscular dystrophies.

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P17.004.C Gene-SCOUT: gene-based biomarker signatures can assist identification of novel genes from phenome-wide association analyses

Lawrence Middleton, *Quanli Wang, Andrew Harper, Abhishek Nag, Dimitrios Vitsios, Slavé Petrovski*

AstraZeneca, Melbourn, Royston, United Kingdom.

Gene-SCOUT (Gene-Similarity from COntinUous Traits) is a gene similarity score that takes a user-defined gene and identifies other human genes with a similar quantitative trait fingerprint. A fingerprint represents the statistical associations arising from studying ~1300 quantitative traits in the UK Biobank 300K exomes. For each pair of genes that shared at least one significant ($p < 1x10^{-5}$) quantitative trait associations we adopted the cosine similarity measure to capture the direction of the effect. Using *APOB* as an exemplar, the top ten ranked genes identified to have the most comparable quantitative trait fingerprint to *APOB* are: ***PCSK9***, *GIGYF1*, ***NPC1L1***, *ZNF229*, ***ANGPTL3***, *RRBP1*, *ACVR1*, *SLC4A1*, ***APOC3*** and ***PDE3B***. The enrichment of **known cholesterol lowering** targets among the top hits demonstrates the robustness of our approach while also suggesting some alternative genes for follow-up. Importantly, Gene-SCOUT provides opportunities to expand our understanding of causal gene networks by identifying genes of similar biology. We accompany the results with a rich web-resource allowing the user to explore in detail similar genes based on a query gene. The web-resource provides a gene network to help visualise and explore genes that are similar to each other according to their fingerprint. In addition, we provide enrichment analyses based on neighbouring genes establishing whether genes close to each other are also enriched for biological processes or UK Biobank disease traits. Gene-SCOUT can facilitate the discovery of novel genes beyond those reported in conventional PheWAS analyses and enables them to be assessed against established disease targets.

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P17.006.A Leveraging auxiliary data from arbitrary distributions to boost GWAS discovery with Flexible cFDR

Anna Hutchinson[1], *Guillermo Reales*[2], *James Liley*[3], *Chris Wallace*[1]

[1]*MRC Biostatistics Unit, University of Cambridge, Cambridge, United Kingdom,* [2]*Cambridge Institute of Therapeutic Immunology & Infectious Disease (CITIID), University of Cambridge, Cambridge, United Kingdom,* [3]*University of Edinburgh, Edinburgh, United Kingdom.*

Genome-wide association studies (GWAS) have identified thousands of genetic variants that are associated with complex traits. However, due to multiple testing constraints GWAS associations must exceed a stringent "genome-wide significance threshold" in order to be considered robust. Consequently, GWAS discovery is currently hampered by the lack of statistical power to detect weaker associations.

The conditional false discovery rate (cFDR) framework provides a tool to leverage one GWAS study to improve power in another. Here, we describe an extension to the cFDR framework, called "Flexible cFDR", that supports auxiliary data from arbitrary distributions whilst controlling the FDR. Our method can be used to iteratively leverage any type of functional genomic data with summary GWAS data to increase power for GWAS discovery, and we introduce an all-encompassing R package to do this, called 'fcfdr' (https://annahutch.github.io/fcfdr/).

We illustrate the flexibility of our method by leveraging GWAS p-values for rheumatoid arthritis, binary data on SNP overlap with transcription factor binding sites and H3K27ac histone modification data in T helper cells, to identify seven newly significant genomic regions that are associated with type 1 diabetes, of which six validated in a larger study. In a second application, we leverage H3K27ac histone modification data in asthma relevant cell types, identifying four significant associations with asthma, of which three validated in the UK Biobank data resource.

Thus, as the already expansive range of functional genomic data grows, our method can exploit this information to increase discovery and provide a more complete picture of genetic associations.

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P17.007.B Software assessment for prioritization of germline causal variants of disease from whole-exome sequencing data

Eva Tosco-Herrera[1], *Alejandro Mendoza-Alvarez*[1], *Hector Rodriguez-Perez*[1], *Adrian Muñoz-Barrera*[2], *Antonio Iñigo-Campos*[2], *Almudena Corrales*[1,3], *Francisco Martinez Bugallo*[4], *Carol Prieto Morin*[4], *Rafaela González-Montelongo*[2], *Jose Miguel Lorenzo-Salazar*[2], *Carlos Flores*[1,2,3], *Laura Ciuffreda*[1], *Itahisa Marcelino-Rodriguez*[1,5]

[1]*Research Unit, Hospital Universitario Nuestra Señora de la Candelaria, Santa Cruz de Tenerife, Spain,* [2]*Genomics Division, Instituto Tecnológico y de Energías Renovables (ITER), Santa Cruz de Tenerife, Spain,* [3]*CIBER de Enfermedades Respiratorias, Madrid, Spain,* [4]*Clinical Analysis Service, Hospital Universitario Nuestra Señora de la Candelaria, Santa Cruz de Tenerife, Spain,* [5]*Instituto de Tecnologías Biomédicas (ITB), Universidad de La Laguna, Santa Cruz de Tenerife, Spain.*

Introduction: Whole-Exome Sequencing (WES) experiments analyze DNA sequences from protein-coding genomic regions, where more than 80% of pathogenic and causal variants of Mendelian diseases are located. Frequently, WES provides a large number of variants per sample (15,000-25,000 variants). Despite causal variant prioritization is necessary for clinical diagnosis, bioinformatic standards are lacking. Here we benchmarked free software tools for variant prioritization of causal variants from empirical WES data.

Materials and methods: A total of 62 WES data from patients with a known genetic diagnosis were obtained using a HiSeq 4000 Illumina® system, using 75 bp paired-end reads and a 100 bp padding to capture the exon-flanking regions. Consistent quality controls and BWA-GATK v3.8 Best Practices were followed for germline variant identification using the GRCh37/hg19 human genome as reference. Whenever necessary, the Human Phenotype Ontology terms were manually inspected according to the declared phenotypes. Different parameters were considered for the performance assessment of nine software tools.

Results and conclusions: Five of the tools were fully evaluated, because of technical limitations and installation issues in the others. Based on conservative first-gene rankings, the highest diagnostic yield was found for Exomiser (69.3%), followed by AMELIE (46.7%), and Tapes (19.3%). In conclusion, the tools for variant prioritization provided largely different diagnostic yields, with Exomiser being the best performing tool. **Funding:** Ministerio de Ciencia e Innovación (RTC-2017-6471-1; AEI/FEDER, UE); ITER agreement OA17/008; FIISC (FIIS19/48); ACIISI (TESIS2020010002); Instituto de Salud Carlos III (CD19/00231); ECIT CGIEU0000219140.

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P17.009.D Need for speed in whole-genome sequencing data analysis: Benchmarking the new generation of alignment and variant calling tools

Sylvan M. Caspar[1,2], *Patricia Stoll*[1], *Janine Meienberg*[1], *Gabor Matyas*[1,3]

[1]*Center for Cardiovascular Genetics and Gene Diagnostics, Foundation for People with Rare Diseases, Schlieren-Zurich, Switzerland,* [2]*Laboratory of Translational Nutrition Biology, ETH, Schwerzenbach, Zurich, Switzerland,* [3]*Zurich Center for Integrative Human Physiology, University of Zurich, Zurich, Switzerland.*

Introduction: Large datasets of whole-genome sequencing (WGS) require fast read alignment and variant calling pipelines. However, the current *de facto* standard pipeline BWA/GATK is hampered by long runtime, warranting faster alternatives with comparable accuracy (sensitivity/precision). Here, we benchmark novel ultra-fast WGS data analysis pipelines.

Materials and Methods: Alignment and variant calling were performed for publicly-available reference datasets (HG001-HG004), while measuring runtime, as well as for ~50 in-house WGS samples (60× PCR-free, PE150) using BWA/GATK, DRAGEN, GENALICE, Isaac/Strelka2, Parabricks (GPU-accelerated BWA/GATK and BWA/DeepVariant), and Sentieon (CPU-accelerated BWA/GATK and BWA/DNAscope). For the reference datasets, sensitivity and precision were assessed using the GIAB truth sets (v.4.2.1) according to the GA4GH guidelines. For our in-house samples, we performed benchmarking on reportedly (likely) pathogenic HGMD/ClinVar variants, enabling performance assessment for sequence variants with potential clinical relevance.

Results: We show that accelerated alignment and variant calling tools reach similar or even better results than standard BWA/GATK (sensitivity/precision >0.999 in high-confidence regions), with runtimes decreased by a factor of ~17×, 24×, 25×, ~53×, and ~84× for Parabricks, Sentieon, Isaac/Strelka2, DRAGEN, and GENALICE, respectively. Furthermore, despite high overall concordance, our data revealed considerable differences in the calling of (likely) pathogenic HGMD/ClinVar variants.

Conclusions: Accelerated alignment and variant calling tools represent significantly faster alternatives to standard BWA/GATK for the primary (GRCh37 or GRCh38) or secondary (e.g. transition to GRCh38) analyses of ever-growing WGS data. However, caution is needed regarding variant calling performance, particularly in the detection of (likely) pathogenic HGMD/ClinVar variants which should not be missed in clinical WGS.

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P17.010.A Mutation showers and mutation fog patterns arise via different APOBEC3 mechanisms in tumors

David Mas-Ponte[1], *Fran Supek*[1,2]

[1]*IRB Barcelona - Institute for Research in Biomedicine, Barcelona, Spain,* [2]*Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.*

The study of the somatic mutations in human tumors has led to the characterization of a diverse set of mechanisms which can create multiple genetic changes in a single event. The activity of APOBEC3 (A3) mutagenic enzymes is the major contributor to these clustered events and is known for its striking mutation showers (kataegis) occurring during the repair of DNA break-points. However, the mechanisms underlying the overall A3 mutation burden, which can be considerable in lung, breast, bladder, and head-and-neck cancers, are less understood.

We systematically measured and classified the diverse patterns of clustered mutations in tumors focusing on APOBEC activity. We identified a new pattern ("omikli", greek for fog) of non-recurrent and diffuse hypermutation which is highly predictive of the overall mutagenic activity of this enzyme in a cancer, and the burden of cancer driver mutations.

This mechanism is independent of that underlying kataegis, and generates short clusters of 2 to 4 mutations. While kataegis predominantly results from the activity of the APOBEC3B paralog, omikli results from the APOBEC3A paralog.

Our data suggests an association with the activity of mismatch repair (MMR) machinery which can provide the single stranded DNA needed as a substrate for the A3 activity. Due to this association, mutations coming from the omikli mechanism accumulate in the early-replicating and gene-rich parts of the genome. Thus, APOBEC mutagenesis has a high propensity to generate impactful, gene-altering mutations, exceeding other well known carcinogens such as tobacco smoking and UV-light.

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P17.012.C Using SMASH for the detection of cancer cells

Sibylle Wilfling[1,2,3], *Sarah M. Hücker*[4], *Jens Kunze*[4], *Claudio Lottaz*[1], *Christoph A. Klein*[4], *Stefan Kirsch*[4]

[1]*Institute of Functional Genomics, Statistical Bioinformatics, University of Regensburg, Regensburg, Germany,* [2]*Center for Human Genetics, Regensburg, Germany,* [3]*Department of Neurology, Bezirksklinikum Regensburg, University of Regensburg, Regensburg, Germany,* [4]*Division of Personalized Tumor Therapy, Fraunhofer Institute for Toxicology and Experimental Medicine, Regensburg, Germany.*

Introduction: Chromosomal instability is one of the hallmarks of cancer and usually detected by expensive and time-consuming array CGH or low coverage whole genome sequencing. The demand for a cheap, flexible-resolution and reliable method for the detection of single cancer cell CNV aberrations is increasing. SMASH (Short Multiply Aggregated Sequence Homologies, Wang et al., 2016) enables genomic copy number variation identification and seems to be a promising method to solve this problem.

Materials and Methods: Based on a cost-effective NGS method previously established by Fraunhofer ITEM, we developed an improved and flexible SMASH algorithm to determine high-resolution CNV profiles of single cells from a breast cancer (SKBR3) and a lymphoblast cell line (GM12878).

Results: Our SMASH version detected larger CNV aberrancies (up to 500.000 bins/genome with mean bin length of 5.256 bp) and distinguished breast cancer cells from the lymphoblastic cells in a fast (runtime about one hour), inexpensive (WGS coverage required: less than 0.7) and reliable (high concordance between cells from the same cell line, good concordance with results from PacBio sequencing) way with flexible resolution.

Conclusions: SMASH is suitable for the detection of CNV changes and thus can be used to discriminate between cancer

cells with genomic instability and normal cells at very low coverage. Higher coverage and resolution allow more detailed characterization of copy number gains and losses. In the future, SMASH might be able to diagnose cancer e. g. from circulating tumor cells in a simple blood sample.

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P17.013.D A cancer genomics reference resource and implementation toolkit around GA4GH standards

*Qingyao Huang[1], Bo Gao[1], Rahel Paloots[1], Paula Carrio-Cordo[2], Ziying Yang[1], **Michael Baudis**[1]*

[1]University of Zurich, Zürich, Switzerland, [2]Paula Carrio Cordo, Zürich, Switzerland.

The Progenetix oncogenomics resource provides sample-specific cancer genome profiling data and biomedical annotations as well as provenance data for cancer studies. Especially through more than 100k genomic copy number number (CNV) profiles from over 500 cancer types, Progenetix empowers comparative analyses vastly exceeding individual studies and diagnostic concepts.

Progenetix has been used in research studies, clinical diagnostics and in the development of data standards for the Global Alliance for Genomics and Health (GA4GH) and the European bioinformatics initiative ELIXIR. The Beacon+ service, implemented on top of Progenetix data, has been instrumental in developing and testing features of the Beacon protocol such as structural variant queries, "handover" data delivery and the implementation of queries for phenoptypic and diagnostic parameters for the upcoming Beacon v2 protocol. During development of GA4GH metadata concepts and schemas - which influenced standards such as the Phenopackets format - cancer specific annotations from Progenetix have informed conceptual requirements and domain-specific mappings. The resource's focus on structural genome variants has been instrumental in addressing their specific requirements in GA4GH schema development and the Beacon protocol.

We demonstrate how an open genomic reference resource has been built around emerging GA4GH standards and how it is being used to support ongoing and future developments in GA4GH and ELIXIR implementation studies, including an introduction about utilizing the Progenetix code repositories for genomics resource development. Highlights from the aggregation of cancer genomic profiling data and the associated annotations will be presented.

progenetix.orggithub.com/progenetix

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P17.014.A Matching cell lines with cancer type and subtype of origin via mutational, epigenomic, and transcriptomic patterns

***Marina Salvadores**[1], Francisco Fuster-Tormo[1,2], Fran Supek[1,3]*

[1]Institute for Research in Biomedicine (IRB), Barcelona, Spain, [2]Institut de Recerca Contra la Leucèmia Josep Carreras, Badalona, Spain, [3]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.

Introduction: Human-derived cancer cell lines are widely used as tumor models, for which their tissue-of-origin provides biological context. However, not all cell lines are suitable as models and those that better resemble the biological and molecular characteristics of the original cancer should be prioritized.

Materials and Methods: We used gene expression and DNA methylation profiles from 9039 human tumors to generate 16 cancer type classifiers. We applied them to 614 cell lines obtaining a resemblance score for each cancer type. Then, we searched for associations between drug response and biomarkers using the original cancer type labels versus taking into account the new cancer type predictions.

Results: We found 366 (60%) cell lines whose both transcriptomic and epigenomic profiles strongly resemble their cancer type-of-origin. For these cell lines we provided tentative subtypes. Surprisingly, we identified 35 (6%) cell lines that better matched a different tissue type than the one they were originally annotated with, both by transcriptome and epigenome. Accounting for these new labels in cell line panels (i.e. removing suspect cell lines) changed the interpretation of drug screening association studies, revealing previously unknown genomic determinants of sensitivity or resistance.

Conclusions: When selecting a panel of cell lines for experimental work, we suggest that those better resembling the tissue-of-origin by the transcriptomic and epigenomic patterns should be prioritized. This work was recently published as Salvadores et al. (2020) Science Advances, DOI: 10.1126/sciadv.aba1862. Funding: ERC Starting Grant, FPU fellowship, ICREA professorship and IRB core funding.

M. Salvadores: None. **F. Fuster-Tormo:** None. **F. Supek:** None.

P17.015.B Nucleosome positioning based identification of tissue contributions in cell-free DNA

***Sebastian Röner**[1,2], Martin Kircher[1,2]*

[1]Charité Universitätsmedizin Berlin, Berlin, Germany, [2]Berlin Institute of Health (BIH), Berlin, Germany.

Cell-free DNA (cfDNA) is found in many bodily fluids and is believed to derive primarily from apoptosis of hematopoietic cells. In the context of certain physiological conditions or disease processes, the proportion of tissues contributing to cfDNA changes. These observations led to an increased research interest in cfDNA for so-called liquid biopsies.

Besides tracing genetic alleles and methylation states, past studies showed that cfDNA fragmentation is associated with nucleosome footprints and DNA binding (Snyder et al.,2016). The fragments carry remnants of their cell-type specific epigenome, which can be leveraged to infer tissues-of-origin. We previously prototyped a pipeline based on Windowed Protection Scores and quantification of nucleosome distances from Fast Fourier Transformation. We showed that cfDNA from healthy individuals most strongly correlates with expression of hematopoietic cell-types. In contrast, in samples from late-stage cancer patients the major contributions align with the cancer's tissue-of-origin.

Here, we describe an easy-to-use computational pipeline implemented to identify these major contributions to cfDNA samples (https://github.com/kircherlab/cfDNA). Based on read alignments to GRCh37 or GRCh38, nucleosome-positioning signals around transcribed genes are automatically quantified and correlated with gene expression values of the Human Protein Atlas (Uhlén et al., 2015). The most correlated expression profiles are highlighted for each sample, with the option to contrast them to another sample (e.g. disease vs. control, time points). The workflow is implemented in Snakemake and Python, with all software dependencies managed via conda. With increased scalability and usability, this analysis can now be easily performed on a wide range of cfDNA samples.

S. Röner: None. **M. Kircher:** None.

P17.016.C Cutevariant: a GUI-based desktop application to explore genetics variations

sacha schutz

Université Brest, Brest, France.

Cutevariant is a user-friendly GUI based desktop application for genomic research designed to search for variations in DNA samples collected in annotated files and encoded in the Variant Calling Format. The application imports data into a local relational database wherefrom complex filter-queries can be built either from the intuitive GUI or using a Domain Specific Language (DSL). Cutevariant provides more features than any existing applications without compromising on performance. The plugin based architecture provides highly customizable features. Cutevariant is distributed as a multiplatform client-side software under an open source licence and is available at https://github.com/labsquare/Cutevariant. It has been designed from the beginning to be easily adopted by IT-agnostic end-users.

S. schutz: None.

P17.017.D Automated Deep Learning Software for PCR/Capillary Electrophoresis Fragment Analysis Enables Efficient Pan-Ethnic *CFTR* Testing at Scale

Elliot Hallmark, *Jacob Ashton, Ryan Routsong, Brian C. Haynes, Brad Hall, John Milligan, Gary J. Latham, Jessica L. Larson*

Asuragen, Austin, TX, USA.

Introduction: Cystic Fibrosis (CF) is an autosomal recessive disease associated with mutations in *CFTR*. We have developed a PCR/capillary electrophoresis (CE) assay that detects 67 pathogenic variants including SNPs, INDELs, CNVs, and tandem repeats covering ≥93% of carriers and ≥99% of affected individuals by mutation prevalence determined by large-scale, pan-ethnic population studies. Despite the accessibility of PCR/CE as an assay format, downstream analysis is tedious, manual, and error prone. To overcome these challenges, we developed software powered by deep learning CE analysis algorithms to perform automated sample genotyping and QC interpretation.

Materials and Methods: Over 3600 electropherograms and 100,000 genotype peaks were used to develop automated genotyping methods. Electropherograms were generated by Asuragen's prototype AmplideX® *CFTR* PCR/CE assay using cell lines, blood samples, and synthetic constructs across four CE instruments and different extraction and assay conditions. We trained a deep convolutional neural network to classify peaks within the raw signal and developed assay-specific logic to translate peak calls into sample genotypes. Automated genotyping and QC logic was bundled into push-button reporting software for use with the PCR/CE assay.

Results: Our automated genotyping software achieved performance (>99.5% SNP- & INDEL-level accuracy) on par with trained technicians. For CNVs (e.g. CFTRdele 2,3), we achieved >99% accuracy. Overall analysis time was reduced from over two hours to <10 minutes per 96 samples.

Conclusion: The software demonstrates the potential to run PCR/CE at scale for CF carrier screening and molecular diagnosis in decentralized laboratory settings. The approach is also extensible to other PCR/CE-based assays.

E. Hallmark: A. Employment (full or part-time); Significant; Asuragen. **J. Ashton:** A. Employment (full or part-time); Significant; Asuragen. **R. Routsong:** A. Employment (full or part-time); Significant; Asuragen. **B.C. Haynes:** A. Employment (full or part-time); Significant; Asuragen. **B. Hall:** A. Employment (full or part-time); Significant; Asuragen. **J. Milligan:** A. Employment (full or part-time); Significant; Asuragen. **G.J. Latham:** A. Employment (full or part-time); Significant; Asuragen. **J.L. Larson:** A. Employment (full or part-time); Significant; Asuragen.

P17.018.A Automated prediction of the clinical impact of copy number variants: The power of combining expert and machine learning approach

Jaroslav Budiš[1,2,3], *Tomáš Sládeček*[1], *Marcel Kucharík*[1,2], *Michaela Gáiová*[1,4], *Zuzana Pös*[1,5,6], *Ondrej Pös*[1,5], *Mário Hlavačka*[1], *Martin tevko*[1], *Werner Krampl*[1,5], *Rastislav Hekel*[1,3,5], *Ján Radvánszky*[1,5,6], *Tomá Szemes*[1,5,2]

[1]*Geneton Ltd., Bratislava, Slovakia,* [2]*Comenius University Science Park, Bratislava, Slovakia,* [3]*Slovak Center of Scientific and Technical Information, Bratislava, Slovakia,* [4]*Department of Computer Science, Faculty of Mathematics, Physics and Informatics, Bratislava, Slovakia,* [5]*Department of Molecular Biology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia, Bratislava, Slovakia,* [6]*Institute of Clinical and Translational Research, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia.*

Introduction: Copy number variants (CNVs) play an important role in many biological processes, including the development of genetic diseases, making them attractive targets for genetic analyses. The interpretation of the effect of structural variants is a challenging problem due to highly variable numbers of gene, regulatory, or other genomic elements affected by the CNV. The state-of-the-art scoring scheme proposed by the American Academy of Medical Genetics (ACMG) is a well-respected guideline for the interpretation. The proper evaluation of the scheme is however challenging even for experts skilled in clinical genetics.

Materials and Methods: We automated several steps of the ACMG scoring scheme, proposing clinicians the recommended choices along with enclosed explanatory genomic annotations. In addition, we implemented a novel method based on machine learning that uses its own modeling beyond the ACMG scheme to predict the clinical impact of CNVs.

Results: We demonstrate the high accuracy of the automated scoring of the ACMG scheme and compare it with the accuracy of the machine learning approach. We show that the combination of these two complementary methods accurately predicts the impact of the majority of CNVs extracted from the ClinVar database.

Conclusions: Prediction of the clinical impact of CNVs can be automated, relieving highly valued clinical professionals of tedious annotation and interpretation processes.

J. Budiš: A. Employment (full or part-time); Significant; Geneton Ltd. **T. Sládeček:** A. Employment (full or part-time); Significant; Geneton Ltd. **M. Kucharík:** A. Employment (full or part-time); Significant; Geneton Ltd. **M. Gáiová:** A. Employment (full or part-time); Modest; Geneton Ltd. **Z. Pös:** A. Employment (full or part-time); Modest; Geneton Ltd. **O. Pös:** A. Employment (full or part-time); Modest; Geneton Ltd. **M. Hlavačka:** A. Employment (full or part-time); Modest; Geneton Ltd. **M. tevko:** A. Employment (full or part-time); Modest; Geneton Ltd. **W. Krampl:** A. Employment (full or part-time); Significant; Geneton Ltd. **R. Hekel:** A. Employment (full or part-time); Significant; Geneton Ltd. **J. Radvánszky:** A. Employment (full or part-time); Modest; Geneton Ltd. **T. Szemes:** A. Employment (full or part-time); Significant; Geneton Ltd..

P17.019.B MGvizCNA: a precision medicine webapp with CNA evidence scoring

*Pedro Pons-Sunyer[1], Jose M. Juanes[1], David Gomez-Peregrina[2], Daniel Perez-Gil[3], Rodiel Martinez-Jimenez[1], Juan M. Rosa-Rosa[4], Maria Ajenjo-Bauza[1], Pablo Cano-Jimenez[1], Daniel Gil-Conejo[1], Jaime Moreno-Martinez[1], Cesar Serrano[5], Ana Barbara Garcia-Garcia[6], **Pablo Marin-Garcia**[7]*

[1]mgviz.org, Valencia, Spain, [2]Sarcoma Translational Research Laboratory (VHIO), Vall d'Hebron, Spain, [3]University of Cambridge, Cambridge, United Kingdom, [4]Haematological Malignancies Clinical Research Unit, H12O-CNIO, Valencia, Spain, [5]Sarcoma Translational Research Laboratory (VHIO), Vall d'Hebron, Spain, [6]CIBER de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM). Unidad de Genómica y Diabetes, INCLIVA, Valencia, Spain, [7]Kanteron systems, Paterna, Spain.

Background: Copy Number Alterations (CNA) play an important role in cancer and mendelian diseases. The current CNA detection methodologies using NGS are based mainly on genome reads count coverage and its profile. Usually, read counts present random noise and biases, which need to be mitigated in order to accurately analyze copy numbers. Currently there is no consensus yet for accurately calling CNAs, resulting in many different tools calling too many CNV candidates with little concordance between their results. Therefore there is a need for exploring analytical consensus scoring and interactive visualization tools in order to extract the right clinical information from the data.

Description: MGvizCNA has its own CNA caller based on wavelet shrinkage and total variation denoising to smoothen readcount data while preserving high frequency information such as breakpoints, and uses a principal component analysis and expectation-maximization based technique to reduce undesired biases between samples. Log2ratio segmentation is done via CBS and fused lasso techniques. MGvizCNA includes a CNVReporter application for interactive visualization and reporting.

Conclusions: MGvizCNA features a machine learning layer to clusterize a given sample with similar individuals and detect CNAs as anomalies that deviate from the assigned population. A workflow for CNA assessment and a web application for exploring and annotating the complexity of CNA detection has been created, along by a decision support system for curating and summarizing CNAs from different callers and helping to describe alteration patterns found in large datasets associated with different pathologies.

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P17.020.C SavvyContaminationFinder:Identifying the source of contamination inshort readsequence data

***Matthew N. Wakeling**, Rebecca Ward, Sarah E. Flanagan, Sian Ellard, Andrew T. Hattersley*

University of Exeter Medical School, Exeter, United Kingdom.

Introduction: Contamination of human DNA samples with DNA from a different human has the potential to confound results, with missed true variants, homozygous variants mis-called as heterozygous, and false positive calls. A robust approach to contamination involves both prevention (using stringent laboratory procedures) and detection (using bioinformatic tools). Any samples with detected contamination should be re-sequenced, after the root cause of the contamination is identified and corrected. However, without identifying the origin human that provided the DNA contaminant, this can be difficult.

Materials and Methods: We developed SavvyContaminationFinder, which can analyse VCF files containing variant calls from multiple samples, and identify which (if any) of the samples are the source of the contamination in a sample. The technique works on short read sequence data from small targeted gene panels up to whole genome sequencing.

Results: We tested the software on samples with contamination added in-silico, and determined that the software correctly identifies the source and quantity of contamination, even when multiple contaminants are used. We analysed a batch of 26 whole genome samples where 22 were accidentally contaminated, with contamination levels ranging from 1.9% to 30%. Results showed that when arranging the samples in rows of 8 in the order specified in the sample list, the contaminant was always the neighbouring sample, indicating a robotics issue in sample handling.

Conclusions: SavvyContaminationFinder allows the source of contamination to be determined from short read sequence data. This enables incident response, root cause analysis, and correction of issues causing contamination.

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P17.021.D Deploying mouse resources for COVID-19 and related coronavirus research at Mouse Genome Informatics

***Anna V. Anagnostopoulos**, Susan M. Bello, Monika Tomczuk, David R. Shaw, Cynthia L. Smith, Carol J. Bult, The Mouse Genome Informatics Staff and Software Team*

The Jackson Laboratory, Bar Harbor, ME, USA.

The ongoing pandemic caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) emphasizes the urgent need for preclinical mouse models to study SARS-CoV-2 biology and pathogenesis and evaluate candidate vaccines and therapeutics against COVID-19. As conventional laboratory mice are inherently resistant to SARS-CoV-2 infection, various strategies have been adapted to deploy infection-permissive mouse models with a range of viral tropisms and COVID-19 clinical features. These include: transgenic and knock-in mouse strains expressing human angiotensin-converting enzyme 2 (hACE2) under heterologous gene promoters or the endogenous mouse *Ace2* promoter; and common inbred strains transduced with hACE2-encoding adenoviral/adenoviral-associated vectors or infected with mouse-adapted SARS-CoV-2 strains. Improved model designs will leverage select mouse genetic tools and strains in the context of age, gender or comorbidities to replicate pulmonary, immunopathological or systemic hallmarks of severe COVID-19 and explore genetic/other modifiers of susceptibility, progression and outcome.

In keeping with its mission as the core knowledgebase for mouse-human comparative biology, Mouse Genome Informatics (MGI, www.informatics.jax.org) has implemented a Coronavirus Information portal to streamline access to current mechanistic and therapeutic mouse studies of COVID-19. The portal aggregates mouse research resources for SARS-CoV-2 and other coronaviruses, including curated publications and preprints, new and repurposed mouse models, and human/mouse genes implicated in coronavirus infection pathophysiology. We present recent enhancements including: focused expansion of Mammalian Phenotype ontology terms enabling enhanced and refined retrieval of coronavirus-relevant model phenotypes and genes; a redesigned page display; and sort/filter functionality facilitating

analysis of customized datasets by model design or research application. Supported by NIH grant HG000330.

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P17.022.A Exploring a strategy for the development of AI-based diagnostic tools for rare diseases

Paula Romero-Campo[1], *Bertha Guijarro-Berdiñas*[1], *María Jesús Sobrido*[2], *Amparo Alonso-Betanzos*[1]

[1]*Research Center on Information and Communication Technologies (CITIC), Universidade da Coruña, A Coruña, Spain,* [2]*Instituto de Investigación Biomédica A Coruña (INIBIC), A Coruña, Spain.*

Background: Developing Artificial Intelligence (AI) -based tools for rare genetic disorders implies grasping the key elements of medical thinking for differential diagnosis. For that purpose, instead of using all available knowledge and data sources, pilot applications may benefit from a reductionist approach.

Aim: To build a schematic scaffold database for autosomal recessive genetic ataxias and spastic paraplegias (ASPs) that can be used to pilot test an automatic diagnostic algorithm.

Methods: We selected a list of ASP entities following the indications of expert neurologists, collected the most characteristic terms for each entity automatically from OMIM and ORPHANET, and we curated the list through expert review. We translated into English two clinical cases, consisting of several medical records spanning many years. Then, we extracted key terms, mapped them to HPO when possible, and checked them against our repository to test our method.

Results: We built a pilot database with around 100 recessive ASPs. We identified an average of 19.3 characteristic terms per entity. We were able to find HPO identifiers for 60% of the terms. We detected some terms lacking discriminative power for the diagnosis, since they appeared in almost half of the entities studied. In contrast, 253 terms corresponded to a single entity. Finally, 25% of symptoms in patient data were matched.

Conclusion: Compiling a large knowledge base regarding rare disorders into a reduced database of characteristics influencing differential diagnosis can be effective to develop computerized diagnostic aids. Additionally, applying these methods to real medical data demands a previous formalization phase.

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P17.023.B DIVAs: a phenotype-based machine-learning model to assess the pathogenicity of digenic variant combinations

Federica De Paoli[1], *Ivan Limongelli*[2], *Susanna Zucca*[2], *Federica Baccalini*[1], *Valentina Serpieri*[3,4], *Fulvio d'Abrusco*[4], *Marina Zarantonello*[5], *Giovanna Fabio*[6], *Maria Carrabba*[6], *Enza Maria Valente*[3,4], *Paolo Magni*[1]

[1]*Department of Electrical, Computer and Biomedical Engineering, University of Pavia, Pavia, Italy,* [2]*enGenome srl, Pavia, Italy,* [3]*IRCCS Fondazione Istituto Neurologico Nazionale Casimiro Mondino, Pavia, Italy,* [4]*Department of Molecular Medicine, University of Pavia, Pavia, Italy,* [5]*Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy,* [6]*Internal Medicine Department, Primary Immunodeficiencies Centre, Fondazione IRCCS Ca' Granda Ospedale Maggiore, Milan, Italy.*

Background: For decades, the inheritance mechanism of genetic disorders was explained through the "*one gene-one disease*" paradigm. Recently, more complex models were proposed to explain those genetic disorders not solvable through a single causative mutation. Here, we propose a method (DIVAs) to assess variants combination pathogenicity in support of digenic inheritance.

Methods: DIVAs is a machine learning model developed to classify combinations of digenic variants with respect to patient's phenotypes (provided as HPO terms). Model features capture all characteristics of a variant combination and its association with patient's phenotypes at variant, gene and gene-pair level. The algorithm was trained and tested on a dataset of validated pathogenic (http://dida.ibsquare.be) and benign variants combinations (https://www.internationalgenome.org/).

Results: DIVAs identified 64 on 78 digenic combinations when validated on independent datasets from literature review. Moreover, the algorithm was employed to identify the pathogenic digenic variants from WES data of three clinical cases for which a single causative mutation was not able to explain their complex traits. Firstly, two male siblings with complex phenotypes such as microcephaly, intellectual disability and seizures: DIVAs identified the variant combination involving FRMPD4 and PAK3 genes among all candidate combinations. Secondly, a patient diagnosed in adulthood for severe combined immunodeficiency "leaky" phenotype, showed a pathogenic variant combination involving CARD11 and STAT3 genes that was successfully ranked by DIVAs in the top five. In conclusion, our method can contribute to uncover the missing heritability of genetic disorders by testing the digenic hypothesis and can be further expanded to oligogenic variant combinations.

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P17.024.C Investigation of the role of long non-coding RNAs in esophageal squamous cell carcinoma

Aigul Sharip[1], *Saule Rakhimova*[1], *Mukhtar Tuleutaev*[2], *Baktybai Orazbekov*[2], *Askhat Molkenov*[1], *Ulan Kozhamkulov*[1], *Yuri Zhukov*[2], *Marat Omarov*[2], *Ainur Akilzhanova*[1], *Ullykbek Kairov*[1]

[1]*National Laboratory Astana, Nur-Sultan, Kazakhstan,* [2]*Multidisciplinary Medical Center of the akimat of Nur-Sultan city, Nur-Sultan, Kazakhstan.*

Esophageal cancer is one of the most common types of cancer worldwide and sixth in Kazakhstan. Esophageal squamous cell carcinoma (ESCC) is highly aggressive with poor survival rate. Long non-coding RNAs (lncRNAs), transcripts longer than 200 nucleotides, play important roles in tumorigenesis and lncRNAs are also involved in the development and progression of ESCC. The aim of the study was to identification of lncRNAs from whole-transcriptome sequencing of Kazakhstani patients and understanding their role in pathogenesis of disease. Tissue samples were obtained from 25 ESCC-affected individuals immediately after Ivor-Lewis esophagectomy from Oncology Center in Nur-Sultan. STAR software and DESeq2 package were used for mapping and defining differentially expressed genes. The lncRNAs have been identified from the list of differently expressed genes (DEGs) among tumor and normal esophageal tissues. The study sized 14 men and 11 women, 88% of the patients were diagnosed with advanced stages T3-T4. Among 1197 DEGs (with adjusted p-value <0.05), we found 61 lncRNAs, comprising 59 upregulated and 2 downregulated genes. Identified lncRNAs are potentially novel and have not been previously described, so the involvement of these lncRNAs in ESCC pathogenesis will be studied using functional and enrichment analysis. The recent studies reveal that lncRNAs may actively associate with the pathogenesis of ESCC. The implications of novel lncRNAs for pathogenesis and

development of potential diagnostics will be further studied.Work was supported by grant of the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan, #AP09058660, and NU CRP grant 021220CRP2222.

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P17.025.D ILIAD project: the ERN-ITHACA online registry of rare diseases with intellectual disability and anomalies of development

Morris Swertz[1], *Klea Vyshka*[2,3], *Fernanda de Andrade*[1], *Joeri Van Der Velde*[1], *Lennart Johansson*[1], *Dieuwke Prins*[1], *Jill Clayton-Smith*[4,5], *Dorica Dan*[6], *Sofia Douzgou Houge*[4,7], *Laurence Faivre*[8,9], *Tiziana Franchin*[10], *Andrew Green*[11], *Raoul Hennekam*[12], *Anne Hugon*[2], *Sylvia Huisman*[12], *Helger Ijntema*[13], *Tjitske Kleefstra*[14], *David Koolen*[14], *Andrea Manunta*[15], *Jukka Moilanen*[16], *Giovanni Mosiello*[17], *Sarra Selatnia*[2], *Mahsa Shabani*[18], *Ammi Sundqvist*[19], *Marco Tartaglia*[20], *Zeynep Tümer*[21], *Birute Tumiene*[22], *Lisenka Vissers*[13,23], *Dagmar Wieczorek*[24], *Giuseppe Zampino*[25], *Alessandra Renieri*[26,27,28], *Alain Verloes*[2,29]

[1]*Dept. of Genetics, Genomics Coordination Center, University Medical Center Groningen, Groningen, Netherlands,* [2]*Dept. of Genetics, Assistance Publique-Hôpitaux de Paris - Université de Paris, Paris, France,* [3]*CERCRID, UMR 5137 "Centre de Recherches Critiques en Droit", Université de Lyon, Lyon, France,* [4]*Manchester Centre for Genomic Medicine, University of Manchester, Manchester, United Kingdom,* [5]*Manchester Centre for Genomic Medicine, Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom,* [6]*Romanian National Alliance for Rare Diseases RONARD, Zalau, Romania,* [7]*Avdeling for medisinsk genetikk, Haukeland universitetssjukehus, Haukeland, Norway,* [8]*UFR Des Sciences de Santé, INSERM-Université de Bourgogne UMR 1231 GAD « Génétique des Anomalies du Développement », FHU-TRANSLAD, Dijon, France,* [9]*Dept of Genetics and Centres of Reference for Development disorders and intellectual disabilities, FHU TRANSLAD and GIMI Institute, University Hospital Dijon, Dijon, France,* [10]*Ospedale Pediatrico Bambino Gesù, IRCCS, Rome, Italy,* [11]*Department of Clinical Genetics, Temple Street Children's University Hospital, Dublin, Ireland,* [12]*Department of Pediatrics, Academic Medical Centre, Amsterdam UMC, Amsterdam, Netherlands,* [13]*Dept of Human Genetics, Radboudumc, Nijmegen, Netherlands,* [14]*Department of Human Genetics, Radboud University Medical Center, Nijmegen, Netherlands,* [15]*Department of Urology, University of Rennes, Rennes, France,* [16]*Department of Clinical Genetics, Oulu University Hospital, Medical Research Center Oulu, Oulu, Finland,* [17]*Department of Surgery, Urology and Neuro-Urology, Bambino Gesù Pediatric Hospital, Rome, Italy,* [18]*Faculty of Law and Criminology, Ghent University, Ghent, Belgium,* [19]*RBU International SBH, Solna, Sweden,* [20]*Genetics and Rare Diseases Research Division, Ospedale Pediatrico Bambino Gesù, IRCCS, Rome, Italy,* [21]*Department of Clinical Genetics, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark,* [22]*Vilnius University Hospital Santaros Klinikos, Santariskiu, Vilnius, Lithuania,* [23]*Donders Institute for Brain, Cognition and Behaviour, Radboudumc, Nijmegen, Netherlands,* [24]*Institute of Human Genetics and Anthropology, Heinrich Heine University Düsseldorf, Düsseldorf, Germany,* [25]*Department of Woman and Child Health, Center for Rare Diseases and Birth Defects, Institute of Pediatrics, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy,* [26]*Med Biotech Hub and Competence Center, Dept of Medical Biotechnologies, University of Siena, Siena, Italy,* [27]*Medical Genetics, University of Siena, Siena, Italy,* [28]*Genetica Medica, Azienda Ospedaliero-Universitaria Senese, Siena, Italy,* [29]*INSERM UMR 1141 "NeuroDiderot", Hôpital R DEBRE, Paris, France.*

European Reference Network ITHACA is developing a "meta-registry" called ILIAD, connecting 37 HCPs, databases, and biobanks in 13 countries for patients with dysmorphic/MCA syndromes and/or intellectual disability. Through the ERN-ITHACA's expert and patient participation network, ILIAD is able to provide an infrastructure for diagnosis, highly specialised multidisciplinary healthcare, evidence-based management, and collection of secure patient data. The registry is built on MOLGENIS open-source software, providing flexible rich data structures, user friendly data import and querying, and FAIR interfaces for programmatic data exchange. To support the interoperability, the registry is connected to the European Rare Disease Registry Infrastructure ERDRI), uses the minimal dataset of variables from JRC and uses the common Pseudonymisation Tool (EUPID) to allow the linking of RD patient cohorts. ILIAD consists of 2 components: a central, web-based registry and a network of linked satellite/client registries forming the ERN-ITHACA registry federation. Data is modelled adhering to international interoperability standards from JRC and EJP-RD. In addition to the core registry, ILIAD will include thematic sub-registries of patients with biologically proven monogenic or genomic (chromosomal) diagnoses, under the supervision of ERN-based curation teams. ILIAD has adopted a data access policy, for requesting access to the data, Governance of the Registry, compliance with applicable legal and regulatory requirements on the use of Personal Data. We are well underway to share ERN-ITHACA patient data, yielding high-quality epidemiological insights and expert consensus statements, informing policy decisions that impact RD patients in general and care for ERN-ITHACA patients in particular (EU Health Programme Grant 947617).

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P17.026.A A Flexible and Shared Information Fine-mapping Approach with an application to 33 cardiometabolic traits from a Ugandan cohort

Nicolás J. Hernández[1], *Jana Soenksen*[2,3], *Paul Newcombe*[1], *Manjinder Sandhu*[4], *Inês Barroso*[2], *Chris Wallace*[1,5], *Jennifer Asimit*[1]

[1]*MRC Biostatistics Unit, University of Cambridge, Cambridge, United Kingdom,* [2]*Exeter Centre of Excellence for Diabetes Research (EXCEED), University of Exeter Medical School, Exeter, United Kingdom,* [3]*School of Life Sciences, University of Glasgow, Glasgow, United Kingdom,* [4]*Dept of Epidemiology & Biostatistics, School of Public Health, Imperial College London, London, United Kingdom,* [5]*Cambridge Institute of Therapeutic Immunology & Infectious Disease (CITIID), University of Cambridge, Cambridge, United Kingdom.*

Joint fine-mapping that leverages information between related quantitative traits could improve accuracy and precision over single-trait fine-mapping. Using summary statistics, flashfm (FLexible And SHared information Fine-Mapping) fine-maps

association signals for multiple traits measured in the same sample, borrowing information between them in a Bayesian framework. In addition, we address key challenges that arise in real data: missing trait measurements and related individuals. Simulation studies of two traits measured in a single cohort with varying sample size, varying trait correlation, and varying proportion of missing data from one trait demonstrate that flashfm reduces the set of potential causal variants by 30% compared to single-trait fine-mapping when traits share a causal variant; when there are no shared causal variants flashfm has similar results to single-trait fine-mapping. In fine-mapping signals from 33 cardiometabolic traits in a Ugandan cohort, flashfm resulted in an average SNP group size reduction of 29% in 34% of the regions that had signals for at least two traits, compared to single-trait fine-mapping. Flashfm is able to make use of previously generated single-trait fine-mapping results and is freely available as an R package (https://github.com/jennasimit/flashfm). It is computationally efficient and increases fine-mapping accuracy and resolution at lower cost, and is more feasible than collecting larger samples.

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P17.028.C Resolving complex pathogenic alleles using HiFi long-range amplicon data and a new clustering algorithm

John Harting, *Cheryl Heiner, Ian McLaughlin, Lori Aro, Zev Kronenberg*

Pacific Biosciences, Menlo Park, CA, USA.

Introduction: Many genetic diseases are mapped to structurally complex loci. These regions contain highly similar paralogous alleles (>99% identity) that span kilobases within the human genome. Comprehensive screening for pathogenic variants is incomplete and labor intensive using short-reads or optical mapping. In contrast, long-range amplification and PacBio HiFi sequencing fully and directly resolves and phases a wide range of pathogenic variants without inference. To capitalize on HiFi accuracy we designed a new amplicon analysis tool, pbAA. pbAA can rapidly deconvolve a mixture of haplotypes, enabling precise diplotyping and disease allele classification.

Materials and Methods: In this experiment, we analyzed two sets of gene-pseudogene systems, *GBA* and *CYP*, that are the second and eighth most common carrier disease alleles, respectively. Samples tested were known to have pathogenic variants troublesome to test with standard short-read assays. Co-amplified long-range PCR amplicons were generated for *GBA* (13 kb)/*GBAP1* (16 kb), as well *CYP21A2* (10 kb)/*CYP21A1P* (9 kb). HiFi reads were generated from libraries and consensus amplicons were produced using pbAA. Variants were determined using minimap2 alignments along with a custom SQL database for characterizing and reporting results.

Results: We were able to accurately call all pathogenic variants in the test samples for all replicates, including whole-gene deletions, gene duplication, gene fusions, recombinant exons, and phased compound heterozygotes. In one trio affected by adrenal hyperplasia, three large structural variants were correctly and independently attributed to the parents and proband.

Conclusions: This experiment demonstrates how PacBio HiFi data analyzed with pbAA simplifies targeted disease allele identification.

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P17.029.D Bioinformatic identification of potential biomarekers in chronic obstructive pulmonary disease and their link to lung cancer

Bayan Abdullah Mallah[1], *Babajan Banaganapalli*[1], *Noor Ahmad Shaik*[1], *Ramu Elango*[1], *Jumana Al-Aama*[2]

[1]*King Abdulaziz University, Faculty of Medicine, Genetic Medicine department, Jeddah, Saudi Arabia,* [2]*Princess Al-Jawhara Center of Excellence in Research of Hereditary Disorders (PACER-HD), Jeddah, Saudi Arabia.*

Introduction: Chronic obstructive pulmonary disease (COPD) is a common respiratory disease, characterized by a progressive obstruction of airflow to the lungs. This study aims to identify the shared differentially expressed genes (DEGs) involved in pathogenesis of COPD in blood and lung tissue.

Methods: Two gene expression datasets generated from blood (GSE54837) and tissue (GSE8581) were analyzed to detect shared DEGs. We further explored the hub genes, using protein-cluster network and functional enrichment analysis. The prioritized genes were searched in genome wide association study databases to reveal their association with COPD risk, and were also queried these hub-COPD genes in several cancer expression databases for uncovering their probable role in lung cancer.

Results: In the human lung tissue (E-GEOD-8581), and in the COPD blood (GSE54837) datasets, we identified 691 and 743 DEGs respectively, and 63 shared between them. In protein network of 63 nodes found 12 hub genes with network centrality >18. The functional enrichment analysis of 12 hub gene network clusters reveled that they are involved in inflammation process and protein ubiquitination. These 12 might contribute to the pathogenesis of COPD, furthermore two genes IRAK2 and MECOM displayed a critical role in the development of lung cancer in patient with COPD. In addition, we identified 5/12 of hub COPD genes as prognostic factor in lung cancer.

Conclusion: This study revealed novel role for few genes and shed new light on the molecular mechanisms of COPD and lung cancer pathogenesis, and provide potential novel drug targets for both diseases.

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P17.030.A GenRisk: a tool to derive individual-level gene scores based on the load of rare damaging variants

Rana S. Aldisi[1], *Oleg Borisov*[1], *Emadeldin Hassanin*[1,2], *Andreas Mayr*[3], *Peter Krawitz*[1], *Carlo Maj*[1]

[1]*Institute for Genomic Statistics and Bioinformatics, University Hospital Bonn, Bonn, Germany,* [2]*MeGeno S.A, Esch-sur-Alzette, Luxembourg,* [3]*Institute for Medical Biometry, Informatics and Epidemiology, University Hospital Bonn, Bonn, Germany.*

The inheritance of human traits is usually divided into two major classes, monogenic and polygenic or complex. Monogenic phenotypes are usually rare and with high penetrance and the

phenotypic status is driven by specific mutations. In contrast, complex traits are considered as the result of multiple variants with modest effects, that are distributed over the entire genome. An oligogenic contribution is suggested when both high impact mutations and polygenic effects are expected to contribute the genetic susceptibility together due to the enrichment of functional rare deleterious variants. GenRisk (Comprehensive Gene-based Assessment) is a tool that implements different gene-based scoring schemes to weights for deleteriousness and rareness of variants. The gene-based scores are derived from allele frequency and functional annotations of variants of coding-region. Additional genetic (e.g., polygenic risk scores) and non-genetic risk factors can be integrated in GenRisk to perform gene-based association analyses and model predictions. Here we computed CADD-weighted rare-variants (<1% MAF) based gene-scores for ~50k male samples from UK-Biobank. The analysis of male baldness phenotype in UK Biobank exome data showed a strong association with the Androgen Receptor (AR), a gene that was also found to be associated with the phenotype by GWAS. Code Availability: https://github.com/AldisiRana/GenRisk

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P17.032.C Quantifying the shared genetic effects on the regulation of expression and protein levels using related and unrelated individuals

Théo Dupuis[1], *Jose Manuel Soria*[2], *Juan Carlos Souto*[1], *Angel Martinez-Perez*[2], *Jochen M. Schwenk*[1], *Emmanouil Theophilos Dermitzakis*[3], *Ewan Pearson*[1], *Ana Viñuela*[4], *Andrew Anand Brown*[1]

[1]*University of Dundee, School of Medicine, Dundee, United Kingdom,* [2]*Unit of Genomics of Complex Diseases, Research Institute of Hospital de la Santa Creu i Sant Pau, IIB Sant Pau, Barcelona, Spain,* [3]*Department of Genetic Medicine and Development, University of Geneva Medical School, Geneva, Switzerland,* [4]*. Institute of Genetic Medicine, International Centre for Life, Newcastle University, Newcastle upon Tyne, United Kingdom.*

Molecular experiments can be used to identify the gene/protein which mediates GWAS genetic effects on disease risk. For instance, RNA-seq provides genome-wide quantifications of expression and is used to understand the consequences of GWAS variants. However, it will not explain those that act on regulatory processes downstream of transcription. Studies have found low correlations between expression and protein levels, but these could be due to environmental and technical factors. Here, we consider the extent to which eQTLs have also an immediate effect on translation and quantify the degree to which regulations of expression and protein levels share a genetic architecture. We estimated the genetic correlations of whole-blood gene expression (RNA-seq) and plasma proteins (Olink) using two different study designs: a family dataset from a pedigree produced by the GAIT2 project ($N = 67$, 90 proteins, expression of 16748 genes), and a dataset of unrelated individuals from the DIRECT consortium ($N = 3029$, 452 proteins, 16209 genes). We confirmed the low correlation (rP) between the two molecular phenotypes: the median absolute rP was ~0.10 for the 47 gene/proteins pairs tested in GAIT2 (median |rP| ~0.04, 320 pairs (DIRECT)). However, genetic correlations (rG) were significantly larger (median $|rG|=0.34$, Wilcoxon test $p = 2.9e-8$ (GAIT2), median $|rG|=0.38$, $p = 5.3e-51$ (DIRECT)). One example is RETN, for which $rG = 0.80$, $rP = 0.47$ (GAIT2), and the variant rs62109837 was found to regulate both its expression and protein levels. These results suggest that the low phenotypic correlation between expression and protein levels is mainly driven by environmental and technical factors while most genetic effects are shared.

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P17.033.D New RD-Connect GPAP features implemented in collaboration with Solve-RD, EJP-RD and ELIXIR enable the diagnosis of rare disease patients with previously negative WES/WGS

Leslie Matalonga[1], *Davide Piscia*[1], *Anastasios Papakonstantinou*[1], *Carles Hernández-Ferrer*[1], *Alberto Corvò*[1], *Steven Laurie*[1], *Carles Garcia-Linares*[1], *Raul Tonda*[1], *Ida Paramonov*[1], *Daniel Picó*[1], *Marcos Fernandez-Callejo*[1], *Dylan Spalding*[2], *Thomas Keane*[3], *Hanns Lochmuller*[4], *Rita Horvath*[5], *Holm Graessner*[6], *Alexander Hoischen*[7], *Ivo Gut*[1], *Sergi Beltran*[1]

[1]*CNAG-CRG, Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Barcelona, Spain,* [2]*European Molecular Biology Laboratory, European Bioinformatics Institute, Wellcome Genome Campus, Hinxton, Cambridge, UK., Cambridge, United Kingdom,* [3]*European Bioinformatics Institute, Wellcome Genome Campus, Hinxton CB10 1SD, UK, Cambridge, United Kingdom,* [4]*Children's Hospital of Eastern Ontario Research Institute; Division of Neurology, Department of Medicine, the Ottawa Hospital; and Brain and Mind Research Institute, University of Ottawa, Ottawa, Canada., Ottawa, ON, Canada,* [5]*Department of Clinical Neuroscience, University of Cambridge, Cambridge, UK., Cambridge, United Kingdom,* [6]*Institute for Medical Genetics and Applied Genomics, University of Tübingen, Tübingen, Germany, Tübingen, Germany,* [7]*Department of Human Genetics, Radboud University Medical Center, Nijmegen, the Netherlands, Nijmegen, Netherlands.*

Introduction: The RD-Connect Genome-Phenome Analysis Platform (GPAP, https://platform.rd-connect.eu/) is an IRDiRC recognised resource for diagnosis and gene discovery in Rare Diseases (RD). The interface allows clinical scientists to collaboratively analyse integrated genome-phenome data under controlled access. The GPAP contains pseudonymised data from over 15,000 individuals and is a key resource for Solve-RD (http://solve-rd.eu/), EJP-RD (https://www.ejprarediseases.org/) and the ELIXIR RD Community (https://elixir-europe.org/communities/rare-diseases).

Material and methods: The new features have been implemented on the RD-Connect GPAP, which processes and indexes pseudonymised genome-phenome data submitted by partners from Solve-RD and EJP-RD, among others.

Results: Recent developments facilitate data submission and integration, as well as the analysis and visualisation of the data. These development include a module to collate and export phenotypic information using broadly used standards (e.g. HPO, ORDO, OMIM, GA4GH Phenopackets), a user-friendly tool to create in-silico patient cohorts according to phenotypic and experimental information, and the ability to remotely visualise sequence alignments archived at the European Genome-Phenome Archive (EGA). Furthermore we have added a module providing programmatic access to the GPAP for the automation of genomic analyses. Some of these developments have been used to enable the diagnosis of the first 208 patients within the Solve-RD project.

Conclusion: New developments in the GPAP have contributed to identify hundreds of disease-causing variants in patients with RDs and confirm diagnosis hypotheses through patient matchmaking approaches.

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P17.034.A Systematic and automated genotype-phenotype associations reassessment through ClinVar follow-up

Kevin Yauy[1], *Francois Lecoquierre*[2], *Stephanie Baert-Desurmont*[2], *Detlef Trost*[3], *Aicha Boughalem*[3], *Armelle Luscan*[3], *Jean-Marc Costa*[3], *Vanna Geromel*[4], *Laure Raymond*[4], *Pascale Richard*[5], *Sophie Coutant*[6], *Melanie Broutin*[7], *Raphael Lanos*[7], *Quentin Fort*[7], *Stenzel Cackowski*[8], *Quentin Testard*[1], *Abdoulaye Diallo*[7], *Nicolas Soirat*[7], *Jean-Marc Holder*[7], *Denis Bertrand*[7], *Anne-Laure Bouge*[7], *Sacha Beaumeunier*[7], *Jerome Audoux*[7], *David Genevieve*[9], *Laurent Mesnard*[10], *Gael Nicolas*[6], *Julien Thevenon*[1], *Nicolas Philippe*[7]

[1]*Institute of Advanced Biosciences, Centre de recherche UGA, Inserm U 1209, CNRS UMR 5309, Grenoble, France,* [2]*Normandie Univ, UNIROUEN, Inserm U1245 and Rouen University Hospital, Department of Genetics and Reference Center for Developmental Disorders, Rouen, France,* [3]*Laboratoire CERBA, Saint Ouen L'Aumone, France,* [4]*Laboratoire Eurofins Biomnis, Lyon, France,* [5]*AP-HP, DMU BIOGEM, UF Cardiogénétique et Myogénétique Moléculaire et Cellulaire, UMR_S 1166, Sorbonne Université and ICAN Institute, Hôpital Universitaire Pitié-Salpêtrière, Paris, France,* [6]*Normandie Univ, UNIROUEN, Inserm U1245 and Rouen University Hospital, Department of Genetics and Reference Center for Developmental Disorders, F 76000, Normandy Center for Genomic and Personalized Medicine, Rouen, France,* [7]*SeqOne, Montpellier, France,* [8]*Inserm U1216, Grenoble Institut Neurosciences, GIN, Univ. Grenoble Alpes, Grenoble, France,* [9]*Department of Medical Genetics, Rare Disease, and Personalized Medicine, IRMB, University of Montpellier, National Institute of Health and Medical Research, Montpellier University Hospital Center, Montpellier, France,* [10]*Soins Intensifs Néphrologiques et Rein Aigu, Hôpital Tenon, Assistance Publique - Hôpitaux de Paris-Sorbonne Université, Paris, France.*

Background: Despite the increasing accessibility of genome sequencing, medical interpretation is restricted to the knowledge available at the time of analysis. The increasing knowledge would justify the systematic reevaluation of previous analyses, though limitations for such manual reinterpretation renders this approach impractical.

Methods: Here we report an automated reassessment method of variant pathogenicity and gene-phenotype associations, called Genome Alert!, through data-mining of ClinVar database. This method highlight changes that are likely to impact genetic diagnosis predicated on ClinVar submissions processing by interquartile range outlier detection, reclassification monitoring and agglomerative clustering. A retrospective 2-years multicentric series (2018-2019) of 5.285 consecutive patients screened by targeted or exome sequencing were evaluated. Variant laboratory database or VCF files were queried by genomic positions of Genome Alert!'s clinically potential variant selection.

Results: Retrospective analysis of ClinVar submissions highlighted 107.167 significant changes in variant status and a monthly median of 23 new genes associated with Mendelian diseases between July 2017 and December 2019. Application of our variant monitoring system in 4.929 targeted sequencing for cancer predisposition syndromes indicated 45 potential reclassifications, with 42 changes subsequently expert-validated, and pinpointed 5 previously missed diagnoses. For exome sequencing reanalysis, 75 recently identified in ClinVar morbid genes were used for a selective reanalysis of 356 negatives tests. This strategy flagged 42 potentially pathogenic variants and resulting in one new diagnosis.

Conclusions: The open-source Genome Alert! method (https://genomealert.univ-grenoble-alpes.fr/) could enable the systematic reassessment of genomic data in a clinical routine, thus improving diagnostic yield and robustness in genomic medicine.

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P17.035.B HD Hub: a centralized database and interactive web platform for high-definition likelihood inference

Chenqing Zheng[1], *Ao Lan*[1], *Zheng Ning*[2], *Jie Zheng*[3], *Xia Shen*[1,4]

[1]*Biostatistics Group, State Key Laboratory of Biocontrol, School of Life Sciences, Sun Yat-sen University, GuangZhou, China,* [2]*Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden,* [3]*MRC Integrative Epidemiology Unit (IEU), Medical School, University of Bristol, Bristol, United Kingdom,* [4]*Centre for Global Health Research, Usher Institute of Population Health Sciences and Informatics, University of Edinburgh, Edinburgh, United Kingdom.*

High-definition likelihood (HDL) is a powerful method for estimating genetic correlations between complex traits using genome-wide association study (GWAS) summary statistics. Compared to individual-level genetic data, GWAS summary-level data are easier to acquire and computationally more tractable even for very large sample sizes. However, GWAS summary statistics are typically based on different population references and have different formats, causing it challenging to use HDL to estimate genetic correlations across many different traits simultaneously. Here we introduce HD Hub, a centralized database with hundreds of thousands of heritability and genetic correlation estimates, estimated using HDL based on harmonized summary association statistics for complex traits from LD Hub and UK Biobank (UKBB). HD Hub is a web-based platform that provides interactive and real-time visualizations and analysis of HDL results. Future developments of HDL with different extensions can also be explored via HD Hub.

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P17.037.D Computation of a database of interspersed repeats in coding regions of the human genome

Doan T. L. Vo Ngoc[1,2], *Randy Osei*[1,2], *Katrien Dohr*[1,2], *Catharina Olsen*[2,3], *Katrien Stouffs*[1,2], *Karen Sermon*[4], *Sara Seneca*[1,2], *Alexander Gheldof*[1,2]

[1]Neurogenetics Research Group, Reproduction Genetics and Regenerative Medicine Research Group, Vrije Universiteit Brussel, Brussels, Belgium, [2]Center for Medical Genetics, UZ Brussel, Brussels, Belgium, [3]Brussels Interuniversity Genomics High Throughput core (BRIGHTcore), VUB-ULB, Brussels, Belgium, [4]Department for Reproduction and Genetics, Vrije Universiteit Brussel, Brussels, Belgium.

Introduction: Our group recently detected a deletion of GAA exon 9, which was flanked by two repeat sequences. Based on this, we intend to identify the frequency of similar direct repeats spanning an exonic sequence in the coding sequences of the genome. Our main aim is to create a publicly available repository for this type of interspersed repeats.

Materials and Methods: We scanned the human reference genome for all repeat sequences of a length ranging from 7-20bp, separated by a maximum distance of 1000bp. We selected instances where at least one repeat was found in an exon or when both repeats were flanking an exon. The human genome was queried 4^7 times for 7bp repeats on a high-end 24-core computer running with 36 GB of memory.

Results: We developed a fast pipeline which queries the human genome for instances of interspersed repeats. One scanning cycle of the genome for a single 7bp sequence took 30 seconds, while the gene annotations took 8 seconds. In total, the analysis of 4^7 sequences in the human genome took 15 hours. This resulted in a precompiled dataset of interspersed repeats within coding regions of the human genome.

Conclusions: We here present a dataset for interspersed repeats in coding regions of the human genome. Data can be requested for a single gene or in batches through an R query. This will be made publicly available and we aim to use our pipeline to expand our analysis for genomes of other organisms as well.

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P17.038.A Plasma: a versatile e-learning platform for teaching interactively genomic and genetic data analysis with Jupyter notebooks

Claire Vandiedonck[1], *Pierre Poulain*[2], *Sandrine Caburet*[2]

[1]Université de Paris, INSERM, Centre de Recherche des Cordeliers, F-75006 Paris, France, [2]Université de Paris, CNRS UMR 7592, Institut Jacques Monod, F-75013 Paris, France.

Plasma ("Plateforme d'eLearning pour l'Analyse de données Scientifiques MAssives") allows to interactively teach computational analysis of massive scientific data, providing user-friendly, reproducible and high-performance environments. Our previous experience of teaching genomics was limited by available academic computational resources, restricting studies to unrealistically small datasets. Remote access was not possible and the use of Unix terminal was intimidating for most biology students.

Jupyter notebooks distributed by a JupyterHub were chosen to address these limitations. In collaboration with QuantStack, a company strongly involved in the Jupyter ecosystem, we designed an open-source web-based solution that can be easily deployed on bare-metal or virtual servers, able to deploy numerous, simultaneous and specific analysis environments (supporting any programming languages and versions, and Rstudio), with a simple and intuitive interface using configuration files hosted on Github. Plasma utilizes tljh-repo2docker, a plugin for The Littlest JupyterHub.

A prize-winning first instance of Plasma was set up for teachers and students of the European Master of Genetics (Université de Paris). It was used extensively since september 2020 and enabled about 150 users to carry on bioinformatic training despite the pandemic. Students could connect remotely and carry out their analysis without installing anything on their own device. Two widgets were also developed, ipycytoscape and ipyigv, to use Cytoscape and the genome browser IGV in Jupyter notebooks. A full documentation is available (plasmabio.readthedocs.io).

Thus, Plasma provides an integrated versatile solution to teach in a user-friendly way realistic bioinformatic analyses, thus better preparing students for their future work in research labs.

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P17.039.B LoFTK: a framework for efficient and automated prediction of loss-of-function variants

Abdulrahman Alasiri[1,2], *Konrad J. Karczewsk*[3,4], *Brian Cole*[5], *Bao-Li Loza*[6], *Sander W. van der Laan*[1,7], *Folkert W. Asselbergs*[1,8,9], *Brendan James Keating*[6], *Jessica van Setten*[1]

[1]Department of Cardiology, Division Heart and Lungs, University Medical Center Utrecht, University of Utrecht, Utrecht, Netherlands, [2]Medical Genomics Research Department, King Abdullah International Medical Research Center, King Saud Bin Abdulaziz University for Health Sciences, Ministry of National Guard Health Affairs, Riyadh, Saudi Arabia, [3]Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, MA, USA, [4]Analytic and Translational Genetics Unit, Massachusetts General Hospital, Boston, MA, USA, [5]Bioinformatics Core, Harvard Medical School, Boston, MA, USA, [6]Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA, [7]Central Diagnostic Laboratory, Division Laboratories, Pharmacy, and Biomedical genetics, University Medical Center Utrecht, University of Utrecht, Utrecht, Netherlands, [8]Health Data Research UK and Institute of Health Informatics, University College London, London, United Kingdom, [9]Institute of Cardiovascular Science, Faculty of Population Health Sciences, University College London, London, United Kingdom.

Loss-of-Function (LoF) variants in human genes have drawn attention due to their impact on clinical phenotypes and frequent occurrence in healthy individuals' genomes. The association of LoF variants with complex diseases and traits may lead to the discovery and validation of novel therapeutic targets. Current approaches predict high-confidence LoF variants without identifying the specific genes or the number of copies affected. We have developed the Loss-of-Function ToolKit (LoFTK), which allows efficient and automated prediction of LoF variants from both genotyped and sequenced genomes. LoFTK enables the identification of genes that are inactive in one or two copies and provides summary statistics for downstream analyses. LoFTK accepts input in two standard file formats: VCF and Oxford file format (IMPUTE2 output). Optionally, LoFTK can identify compound heterozygotes if the user supplies phased genotypes. LoFTK generates two tables of LoF variants and their respective genes, listing predicted LoF variants and their zygosity status for each individual, and LoF genes and their inactive copy number for each individual, respectively. In addition, a report with descriptive statistics on the variants and genes is produced. LoFTK is command-line based that allows for integration in existing workflows and enables efficient and automated LoF variant and gene prediction. LoFTK is open source and freely available at https://github.com/CirculatoryHealth/LoFTK.

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P17.040.C miRNA-mRNA regulatory network in testicular tissues with idiopathic Sertoli cell-only syndrome suggest an important role for hsa-miR-122-5p by controlling germ cell development

Jorge Victor Wilfredo Cachay Wester, *Alfredo Ribeiro-Silva, João Monteiro de Pina-Neto*

University of São Paulo, Ribeirão Preto, Brazil.

Introduction: Male factor infertility accounts for about half the cases of couple infertility with genetic and non-genetic causes and in around 50% of cases are idiopathic. The most severe histopathological phenotype in male infertility is the Sertoli cell-only syndrome (SCOS) which is characterized by total germ cell aplasia in testicular tissue. Transcriptome analysis performed formalin-fixed paraffin-embedded (FFPE) of testicular tissue revealed the loss of expression of several genes and microRNAs associated to cell proliferation signaling pathways. However, still lesser is known about the molecular mechanisms involved in idiopathic SCOS. The aim of this study was to report the interaction of hsa-miR-122-5p and its target genes in human male testes with SCOS.

Materials and Methods: For this purpose we collected six FFPE samples of testicular biopsy from men with obstructive azoospermia and Sertoli cell-only syndrome, archived since 2001 and these were analyzed by small RNA-seq and TruSeq-RNA exome in order to detect small non-coding RNA and mRNA expression.

Results: Our analysis detected 37 DE miRNAs and 738 DE mRNA. We found hsa-miR-122-5p upregulated in SCOS samples and its target genes downregulated. Functional annotation of miRNA and mRNA together corresponded to an aberrant expression of genes associated to the cell cycle and meiosis of male germ cells. The regulatory network suggest an important role of hsa-miR-122-5p as post transcriptional regulator of downregulated genes.

Conclusions: We concluded that hsa-miR-122-5p can be associated to the regulation of several genes in human male testes with idiopathic SCOS.

J.C. Wester: None. **A. Ribeiro-Silva:** None. **J. Pina-Neto:** None.

P17.041.D Correction for sample overlap, Winner's curse and weak-instruments bias in two-sample Mendelian Randomization

Ninon Mounier[1,2], ***Zoltán Kutalik***[1,2,3]

[1]*University Center for Primary Care and Public Health, Lausanne, Switzerland,* [2]*Swiss Institute of Bioinformatics, Lausanne, Switzerland,* [3]*Department of Computational Biology, University of Lausanne, Switzerland.*

Introduction: Inverse-variance weighting (IVW) two-sample Mendelian Randomization (MR) is the most widely used method to estimate the causal effect of an exposure on an outcome. However, the resulting causal effect estimates may suffer from different biases due to sample overlap, Winner's curse and weak instruments.

Methods: Assuming a spike-and-slab genomic architecture, we analytically derived the bias of such estimate, which can be quantified using only summary statistics. Hence, we propose a correction of the IVW-MR estimate and compared it against its uncorrected counterpart under a wide range of simulations settings. Finally, we performed IVW-MR based on summary statistics for body mass index (BMI) and systolic blood pressure (SBP) obtained from overlapping samples ($N_{BMI} = 686{,}128$, $N_{SBP} = 340{,}159$, $N_{overlap} = 340{,}159$) and corrected the obtained causal effect estimates using our method.

Results: Using simulated data, we observed that when the confounder and the causal effect are acting in the same direction, IVW-MR effects are overestimated for fully-overlapping samples and underestimated for non-overlapping samples. When they are acting in opposite directions, observed effects are underestimated for all overlaps because the three sources of biases are towards the null. In all the explored scenarios, our correction reduced bias (up to 30 folds). Using summary statistics for real data, our method revealed that IVW-MR causal effects of BMI on SBP and of SBP on BMI were both significantly overestimated (by 15% and 10% respectively).

Conclusions: We developed a method to correct causal effect estimates for sample overlap, weak instrument bias and Winner's curse simultaneously using only summary statistics.

N. Mounier: None. **Z. Kutalik:** None.

P17.042.A Intake of synbiotic alters metabolism, decision-making and behavior

Aakash Mantri

Institute for Genomic Statistics and Bioinformatics, Bonn, Germany.

A higher gut microbial diversity can be associated with improved dietary decisions and healthier choices due to the host being able to evaluate food options for choice and / or retain more (self-) control over his eating decisions. We hypothesized, that a seven-week treatment with a synbiotic dietary supplement, as compared to a placebo treatment, will increase diversity of the gut microbiome in human participants. Furthermore, the modulation of the gut microbiota will alter the human metabolism.Study Design: The data collected were part of a dietary intervention study conducted between March and November 2019 at University Hospital Bonn, Germany. The study was performed with a randomized, placebo-controlled, double-blinded study population. The participants attended two sessions in which behavioral experiments, structural and functional magnetic resonance imaging (MRI) data, psychophysiological as well as metabolic parameters and gut microbiota samples were collected.16S Amplicon sequencing was done on the gut microbiota samples on the 16s region. Sequenced data was analyzed using Qiime2 and phyloseq packages. As a manipulation check of our intervention, we quantified the abundance of the administered beneficial bacteria Lactobacillus and Bifidobacterium, as it is expected to show increased abundance post vs. pre intervention in the intervention compared to the placebo control group. We have compared the change in diversity post versus pre intervention (T2-T1), in the intervention relative to the control group. We also checked for association of all microbiome changes with liver metabolism and behavioral data.

A. Mantri: None.

P17.043.B Comparison of Established Microsatellite Instability Detection Tools in Next Generation Sequencing

Ayça Açar[1], *Merve Say*[1], *Barış Salman*[1], *Mutlu Avşar Dülger*[2]

[1]*Gen Era Diagnostics, Bioinformatics Department, Istanbul, Turkey,* [2]*Gen Era Diagnostics, Application Department, Istanbul, Turkey.*

Repetitive microsatellite sequences are very prone to mutation due to the slippages in homopolymer regions during replication. If the DNA repair system is deflected, these errors cannot be corrected. Thus, the number of these repeat regions varies in population and causes genomic instability. FFPE tumor tissues taken from cancer patients were analyzed with Solid Tumor Solution (STS) kit of Sophia Genetics. Target enrichment was performed with hybrid-capture method and six loci were analyzed. The algorithm compares the homopolymer length at each locus for each sample to an average length of microsatellite. Two different distance scores were calculated; the first one was determined in comparison with the other samples in the same run, whereas the latter was compared to a global database which contains more than 400 clinical samples. The samples with a distance score lower than six were interpreted as microsatellite stable (MSS); whereas the values between six and fourteen indicated microsatellite instability with low confidence (MSI-LC). The samples with scores higher than fourteen were classified as microsatellite unstable with high confidence (MSI-HC). These results were validated with MSIsensor-pro and MANTIS tools with altering the threshold values for classes. In result, unlike the standard PCR and immunohistochemistry methods, next generation sequencing (NGS) allows more than one microsatellite loci to be analyzed more accurately without the need of matched normal tissue. Considering that MSI-high status is becoming a pan-cancer biomarker, using NGS combined with multigene panels will allow for time and cost lowering of biomarker testing in cancer patients.

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P17.044.C Digging exome sequencing data: An example of a homozygous mobile element insertion detected in a rare disease cohort

Philippine Garret[1,2], *Martin Chevarin*[1,3], *Antonio Vitobello*[1,3], *Simon Verdez*[1,3], *Cyril Fournier*[4,5], *Alain Verloes*[6,7], *Emilie Tisserant*[1,3], *Pierre Vabres*[1,8,9], *Orlane Prevel*[1,9], *Christophe Philippe*[1,3], *Anne-Sophie Denommé-Pichon*[1,3,10], *Ange-Line Bruel*[1,3], *Frédéric Tran Mau-them*[1,3,11], *Hana Safraou*[1,3,10], *Aïcha Boughalem*[2], *Jean-Marc Costa*[2], *Detlef Trost*[2], *Laurence Faivre*[1,10], *Christel Thauvin-Robinet*[1,3,11], *Yannis Duffourd*[1,3]

[1]*UMR1231 GAD, Inserm, 21070 Dijon, France,* [2]*Laboratoire Cerba, Saint-Ouen l'Aumône, France,* [3]*Unité Fonctionnelle Innovation en Diagnostic génomique des maladies rares – FHU-TRANSLAD – Dijon University Hospital, Dijon, France,* [4]*University of Burgundy-iSITE – INSERM UMR 1231 – Faculty of Medicine, 21000 Dijon, France,* [5]*Unit for innovation in genetics and epigenetic in oncology – Dijon University Hospital, Dijon, France,* [6]*UMR1141 INSERM – Université Paris Diderot, Paris, France,* [7]*Genetics Department – AP-HP, Robert-Debré University Hospital, Paris, France,* [8]*Centre de Référence maladies rares « maladies dermatologiques en mosaïque » – service de dermatologie – FHU-TRANSLAD – Dijon University Hospital, Dijon, France,* [9]*Service Dermatologie – Dijon University Hospital, Dijon, France,* [10]*Centre de Référence maladies rares « Anomalies du développement et syndromes malformatifs » – centre de génétique – FHU-TRANSLAD – Dijon University Hospital, Dijon, France,* [11]*Centre de Référence maladies rares « déficiences intellectuelles de cause rare » – centre de génétique – FHU-TRANSLAD – Dijon University Hospital, Dijon, France.*

Introduction: About 27% of the human genome is composed of mobile elements (MEs), which can create genomic instability leading to genetic diseases. The mechanism of MEs insertion has been described in several pathologies. It has been estimated that about 0.3% of all variations are due to de novo insertions. Nowadays, the massive development of next-generation sequencing provides the opportunity to identify new MEs insertions on large scale.

Methodology: Exome sequencing (ES) data from patients affected with developmental and/or neurological abnormalities have been analyzed by MELT, a tool identifying MEs insertions. The results were filtered by frequency, impacted region and gene function.

Results: Following phenotype comparison and PCR validation, a convincing candidate was found in a suspected consanguineous patient referred for poikilodermia. A homozygous *Alu* insertion was identified in exon 7 of *FERMT1* gene involved in Kindler syndrome, a condition responsible for poikilodermia. FERMT1 protein mediates anchorage between keratinocytes cytoskeleton and extracellular matrix via focal adhesions. RNA-seq analyzes showed an in-frame exon 7 skipping in proband's fibroblasts. This deletion is located in one of the two FERM domains involved in localizing the protein to plasma membrane. This work provided evidence this Alu insertion is linked to the proband's phenotype.

Conclusion: As two previous studies on ES data, this project aims to identify new insertions in genes and highlights the interest to include MEs detection in ES pipeline to reduce diagnostic wavering. This work on preexisting ES data represents an additional argument in favor of Exome/Genome sequencing suitability as a unique exam.

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P17.046.A Biologically interpretable neural networks for phenotype prediction using population-cohort multi-omics data

Arno van Hilten, *Jeroen G. J. Rooij, Wiro J. Niessen, Joyce B. J. van Meurs, Gennady V. Roshchupkin*

Erasmus MC, Rotterdam, Netherlands.

Background: Multi-omics analysis can provide novel insights into underlying biological mechanisms of traits and complex diseases. However, omics types are often analyzed in separate analyses and combined afterwards. In this study, we propose a novel method for analyzing multiple omics in a single, multivariate analysis, using the flexibility and computational power of neural networks.

Method: In the proposed method, interpretable neural network architectures are constructed by using biological knowledge such as gene annotations and meQTL data to connect gene-expression and methylation data to their corresponding genes (nodes in the network). After training, the resulting architecture allows the extraction of the most predictive CpGs, gene expression levels, and overall genes by inspecting and visualizing the strength of the connections.

Results: As a proof of principle, we applied the method to predict smoking status and subject age in the Rotterdam Study with gene-expression and methylation data from blood ($n = 550$). We achieved an AUC of 0.98 in the held-out test set for smoking status (current smoker vs never smoked) and identified well-replicated genes such as *AHRR*, *GPR15* and *LRRN3*. The network predicted age with a RSME of 4.45 years in the test set and identified the genes *NRCAM, C19orf57* and *MEG1* as most predictive. Additionally, visualization of predictive CpGs demonstrated that dependent CpGs are automatically pruned and that

independent signals can be assigned to biologically regulatory elements.

Conclusion: By using functional genomic annotation data to construct its architecture, we developed an interpretable neural network to analyze multiple omics in a single analysis.

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P17.047.B Diabetes-cancer multi-phenotype GWAS in EPIC study: an improved power for defining genetic causes of multi-morbidity

Ayse Demirkan[1], *Liudmilla Zudina*[1], *Igor Pupko*[1], *Zhanna Balkhiyarova*[1], *Anna Ulrich*[1], *Philippe Froguel*[2,3], *Elio Riboli*[3], *Marika Kaakinen*[1], *Inga Prokopenko*[1,2]

[1]*University of Surrey, Guildford, United Kingdom,* [2]*Institut Pasteur de Lille, CNRS, University of Lille, Lille, France,* [3]*Imperial College London, London, United Kingdom.*

Introduction: There are established relationships between type 2 diabetes (T2D) and cancer. In fact, cancer is the most common cause of death in diabetes. We aimed to gain insights into the pathophysiological processes shared between T2D and four cancers through multi-phenotype (MP) genome-wide association study (GWAS).

Methods: We combined GWAS on 36,173 individuals from the pan-European EPIC study, including 10,855 T2D cases, 4,126 postmenopausal breast, 2,111 colorectal, 473 pancreatic and 419 prostate cancer cases. The combined GWAS dataset was quality controlled and imputed against the HRC reference panel providing 39.3M DNA variants for analysis. We performed reverse regression MP-GWAS as implemented in SCOPA software. We evaluated the driving disease combinations at identified loci using Bayesian information criterion (BIC).

Results: Within MP-GWAS, we identified 450 independent loci ($P < 5x10^{-8}$) for the full five-phenotypes model. Among them, 147 belonged to established T2D/cancer loci. These included rs35011184 (*TCF7L2*) and rs2981578 (*FGFR2*), previously reported for many of five studied outcomes. In our MP-GWAS, these yielded the best model fit for T2D- and breast cancer-only, respectively. The 239 novel signals included rs10497931 at *MAP2* gene ($P = 6.11x10^{-53}$) for the five-phenotype pleiotropic model. Among novel loci, 129 highlighted single-phenotype effect as the best model. The remaining 110 signals showed the best fit for MP model, having 67 of them with T2D and at least one cancer.

Conclusions: The large data and power, improved through implementation of multi-variate GWAS analysis enabled identification of ~1/3 of loci with shared T2D-cancer effects that might contribute to these diseases' comorbidity.

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P17.048.C Multi-omics to predict changes during cold pressor test

Lisette J. A. Kogelman[1], *Madeline Ernest*[2], *Katrine Falkenberg*[1], *Gianluca Mazzoni*[3], *Julie Courraud*[2], *Li Peng*[2], *Susan Svane Laursen*[2], *Arieh Cohen*[2], *Jes Olesen*[1], ***Thomas Folkmann Hansen***[1]

[1]*Copenhagen University Hosital, Glostrup, Denmark,* [2]*Statens Serum Institut, Copenhagen, Denmark,* [3]*Copenhagen University, Copenhagen, Denmark.*

Molecular mechanisms of pain are complex and difficult to entangle, but important to understand to treat pain disorders. The cold pressor test (CPT) is used as pain provocation test in pain research. We hypothesize, that performing multi-omic analyses during CPT gives the opportunity to home in on molecular mechanisms involved. Twenty-two females were phenotypically assessed before and after a CPT, and blood samples were taken. RNA-Sequencing, steroid profiling and untargeted metabolomics were performed. Each 'omic level was analyzed separately at both single-feature and systems-level (e.g. principal component and partial least squares regression analysis) and all 'omic levels were combined using an integrative multi-omics approach, all using the paired-sample design. We showed that unsupervised methods were not able to discriminate time points, while supervised clustering did significantly distinguish time points using metabolomics and/or transcriptomic data, but not using conventional clinical measurements. Transcriptomic and metabolomic data revealed at feature-, systems- and integrative- level biologically relevant processes involved during CPT, e.g. lipid metabolism and stress response. We, therefore, conclude that multi-omics strategies should be exploited in pain research to gain knowledge on the biological mechanisms involved in pain.

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P17.049.D Phenome-wide mantis-ml: automated gene prioritisation across 5,000 diseases with Natural Language Processing

Dimitrios Vitsios, *Ioannis Melas, Andrew Harper, Quanli Wang, Slavé Petrovski*

AstraZeneca, Cambridge, United Kingdom.

Leveraging the information collected from large-scale community endeavours, such as population genomic databases, protein-protein interaction databases and preclinical phenotype databases provides an opportunity for orthogonal evidence to accelerate the identification of novel human genetic associations.

We previously published a novel machine learning method (mantis-ml; *Vitsios et al*., 2020) that showed an ability to prioritise known and novel disease-relevant genes leveraging the biological context of a disease (including pathways, expression and literature) given a set of seed genes for the disease of interest. However, the original mantis-ml implementation required a number of human-dependent manual curation steps, which limited phenotype/disease scalability.

Herein, we present a streamlined, automated and scalable workflow for deploying mantis-ml to thousands of diseases. We achieve this by leveraging Natural Language Processing (NLP) to infer both disease-relevant annotation terms and known disease-associated gene lists. We applied our method across ~5,000 rare-to-common diseases spanning the Human Phenotype Ontology (HPO) and OpenTargets (OT) resources in less than five hours. This phenome-wide mantis-ml resource allows researchers to both identify the top ranked genes associated with a disease of interest (original application) and also to identify the top-ranked human phenotypes for a given gene of interest (extended application). Subsequently, through the mantis-ml predictions, we've constructed a comprehensive atlas of prioritised genes per disease, which is able

to successfully highlight clusters of similar diseases - reinforcing the robustness of the predictions. We accompany our method with a fully interactive web app allowing exploration, visualisation and validation of phenome-wide mantis-ml predictions.

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P17.050.A Multivariate analysis reveals shared genetic architecture of brain morphology and human behaviour

Ronald de Vlaming[1], *Eric Slob*[2], *Philip Jansen*[3], *Philipp Koellinger*[1], *Patrick Groenen*[2], *Niels Rietveld*[2], *Alain Dagher*[4]

[1]*Vrije Universiteit Amsterdam, Amsterdam, Netherlands,* [2]*Erasmus University Rotterdam, Rotterdam, Netherlands,* [3]*Amsterdam UMC, Amsterdam, Netherlands,* [4]*McGill University, Montreal, QC, Canada.*

Human variation in brain morphology and behaviour are related and highly heritable. Yet, it is largely unknown if specific features of brain morphology and behaviour have a shared genetic architecture. Here, we provide estimates of the heritability of grey-matter volume in 74 regions of interest (ROIs) and map genetic correlations between these ROIs and behavioural outcomes. Our results are afforded by a novel method: multivariate genomic-relatedness restricted maximum likelihood (MGREML). This method is optimised both for precision and computational efficiency. We find five genetically distinct clusters in the brain that are aligned with standard anatomical subdivision in neuroscience. Behavioural traits have distinct genetic correlations with brain morphology that suggest trait-specific relevance of ROIs.

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P17.051.B Short tandem repeat detection in next-generation sequencing data

Jeroen Depreeuw[1], *Erika Souche*[1], *Joke Allemeersch*[1], *Wouter Bossuyt*[1], *Kristl Claeys*[2,3], *Philippe Van Damme*[2,4], *Sien Van Daele*[2,4], *Liesbeth De Waele*[5], *Nathalie Goemans*[5], *Els Orbitus*[6,7], *Katleen Ballon*[5], *Hilde Van Esch*[1], *Gert Matthijs*[1], *Sascha Vermeer*[1], *Valérie Race*[1]

[1]*Center for Human Genetics, University Hospital of Leuven, Leuven, Belgium,* [2]*Department of Neurology, University Hospital of Leuven, Leuven, Belgium,* [3]*Laboratory for Muscle Diseases and Neuropathies, Department of Neurosciences, KU Leuven, Leuven, Belgium,* [4]*Department of Neurosciences, Experimental Neurology, and Leuven Brain Institute (LBI), KU Leuven, Leuven, Belgium,* [5]*Department of Pediatric Neurology, University Hospital of Leuven, Leuven, Belgium,* [6]*Center for Developmental Disabilities, University Hospital of Leuven, Leuven, Belgium,* [7]*Department of Development and Regeneration, KU Leuven, Leuven, Belgium.*

Introduction: Short tandem repeats (STRs) are repeated DNA sequences with a length of 3-6 nucleotides. STR expansion disorders are a family of neuropathological disorders linked to the accumulation of STRs and are currently detected with PCR techniques. By applying recent advances in Next-Generations Sequencing (NGS), we investigated the possibility to detect STRs in targeted NGS data.

Materials and Methods: Samples were sequenced on an Illumina HiSeq and NovaSeq (2x125 pair-end). Three targeted capture techniques were selected, including a customized gene panel (n = 732), an exome panel (n = 200) and an exome panel with extra customized probes (n = 20). Expansion Hunter was used to detect 35 relevant STRs and for 8 STRs, results were compared with PCR.

Results: Expansion Hunter could estimate the correct number of STRs with high accuracy (97%) in 8 loci for which PCR data was available. Four samples with known expanded STRs were also flagged with Expansion Hunter. However, full-blown STRs have an upper limit for their repeat estimation due to the probes and insert size. STRs in exome data were sufficiently covered for 20 out of 35 STR regions. Intronic (e.g. C9ORF72) and high GC% STRs (e.g. FMR) were not detected, although adding probes resulted in sufficient coverage for 4 out of 9 intronic STRs.

Conclusions: We were able to successfully validate Expansion Hunter as a tool to detect STR expansions in targeted NGS data. Expansion Hunter results were highly concordant with PCR-based conclusions. However, some regions could not be detected and therefore, results should be interpreted carefully.

J. Depreeuw: None. **E. Souche:** None. **J. Allemeersch:** None. **W. Bossuyt:** None. **K. Claeys:** None. **P. Van Damme:** None. **S. Van Daele:** None. **L. De Waele:** None. **N. Goemans:** None. **E. Orbitus:** None. **K. Ballon:** None. **H. Van Esch:** None. **G. Matthijs:** None. **S. Vermeer:** None. **V. Race:** None.

P17.052.C Bioinformatic NGS data analysis with solida-core

Matteo Massidda[1], *Gianmauro Cuccuru*[2], *Rossano Atzeni*[1], *Rajesh Pal*[1,3], *Paolo Uva*[4,5], *Giorgio Fotia*[1]

[1]*Centre for Advanced Studies, Research and Development in Sardinia (CRS4), Pula, Italy,* [2]*Albert Ludwigs University, Freiburg, Germany,* [3]*University of Cagliari, Cagliari, Italy,* [4]*IRCCS G. Gaslini, Genova, Italy,* [5]*Italian Institute of Technology, Genova, Italy.*

Introduction: We present solida-core (https://github.com/solida-core), an open source collection of reproducible and extensively validated bioinformatic analysis pipelines. The need of reproducible bioinformatic analysis workflows, which are usually weighed down by complex dependency trees and configuration requirements, motivated the development of this resource.

Material and methods: solida-core relies on the Snakemake framework. We defined a "scaffold" for pipeline development, consisting of a section with the analysis steps, named rules, and a configuration file, allowing a simple management of user definable parameters. Pipelines portability is ensured by project-related virtual environments including all required tools and dependencies. Travis CI performs continuous integration, automatically running the test suite whenever the codebase is changed on Github repository.

Results: At the time of writing the collection of pipelines covers DNA, RNA and miRNA data analysis. All the pipelines of solida-core are built, as a first step, following the GATK Best Practices for DNA and RNA sequencing analysis. Further improvements and refinements were then incorporated after their testing with thousands of samples at the CRS4 Next Generation Sequencing Core Facility, one of the largest facilities that operates in Italy.

Conclusions: The solida-core open source collection is publicly released with SOLIDA, a pipeline manager that guides the user

during pipeline configuration and deployment. The combination of these resources represents a complete easy-to-use bioinformatic analysis framework which can be used by both researchers facing bioinformatics for the first time and by experienced bioinformaticians.

M. Massidda: None. **G. Cuccuru:** None. **R. Atzeni:** None. **R. Pal:** None. **P. Uva:** None. **G. Fotia:** None.

P17.053.D GREEN-DB: a framework for the annotation and prioritization of non-coding regulatory variants in whole-genome sequencing

Edoardo Giacopuzzi[1], *Niko Popitsch*[1,2], *Jenny C. Taylor*[1]

[1]*University of Oxford, Oxford, United Kingdom,* [2]*Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), Vienna, Austria.*

Non-coding variants have emerged as important contributors to the pathogenesis of human diseases, not only as common susceptibility alleles but also as rare high-impact variants. Despite recent advances in the study of regulatory elements and the availability of specialized data collections, the systematic annotation of non-coding variants from genome sequencing remains challenging. Here we present a framework for the annotation and prioritization of regulatory variants in WGS/GWAS analysis to support the discovery of candidate disease-associated variants in the non-coding genome. First, we integrated 24 data sources to develop a standardized collection of 2.4 million regulatory elements in the human genome, transcription factor binding sites, DNase peaks, ultra-conserved non-coding elements, and super-enhancers. Information on controlled gene(s), tissue(s) and associated phenotype(s) are provided for regulatory elements when possible. Then, we calculated a variation constraint metric for regulatory regions and showed that genes controlled by constrained regions are more likely to be disease-associated genes and essential genes. Finally, we evaluated 16 non-coding impact prediction scores providing suggestions for variant prioritization. The proposed annotation framework was able to capture previously published disease-associated non-coding variants and its integration in a routine prioritization pipeline increased the number of candidate genes, including genes potentially correlated with patient phenotype, and established clinically relevant genes.

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P17.054.A Penetrance estimation of *SORL1* loss-of-function variants using a family-based strategy adjusted on *APOE* genotypes suggest a non-monogenic inheritance

Catherine Schramm[1], *Camille Charbonnier*[1], *Aline Zaréa*[2], *David Wallon*[2], *Morgane Lacour*[2], *CNRMAJ collaborators, Flora Alarcon*[3], *Emmanuelle Génin*[4], *Dominique Campion*[1], *Grégory Nuel*[5], *Gaël Nicolas*[1]

[1]*Normandie Univ, UNIROUEN, Inserm U1245 and Rouen University Hospital, Department of Genetics and CNR-MAJ, Rouen, France,* [2]*Normandie Univ, UNIROUEN, Inserm U1245 and Rouen University Hospital, Department of Neurology and CNR-MAJ, Rouen, France,* [3]*CNRS 8145-MAP5, Paris, France,* [4]*Inserm U1078, Brest, France,* [5]*CNRS 8001 - LPSM, Paris, France.*

Introduction: For complex disorders, estimating the age-related penetrance associated with rare variants of strong effect is essential. However, rarity and co-occurrence with other risk factors make it difficult to estimate. In the context of Alzheimer Disease, we propose a family-based methodology to estimate the penetrance of *SORL1* rare (allele frequency<1%) loss-of-function variants (LoF, odds ratios >7]) adjusted for *APOE4*, the main risk factor (allele frequency ~14%, odds ratios [3.4-14]).

Material and methods: Our survival model combines: (i) a baseline for non-carriers of SORL1 LoF variants, stratified by *APOE* genotypes derived from a large cohort study and (ii) an additive effect of *SORL1* LoF variants estimated from our family cohort: 34 pedigrees with a proband carrying a *SORL1* protein-truncating or a missense variant with in vitro LoF evidence, onset<75 years, information on relatives (379 phenotypes, 79 genotypes). We embedded this survival model into an Expectation-Maximisation (EM) algorithm to accommodate for missing genotypes. Confidence intervals were computed by bootstrap. To correct for ascertainment bias, proband phenotypes were omitted.

Results: We provide penetrance estimation curves associated with *SORL1* LoF variants at the digenic level. By age 75, we estimate *SORL1* LoF variants to reach a 100% penetrance only among homozygous *APOE4* carriers (75% among *APOE4* heterozygous carriers and 30% among *APOE33* genotype carriers).

Conclusion: This method could be applied to other diseases where penetrance estimates are required to help clinicians in genetic counselling or preventive treatment strategies.

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P17.055.B Improved estimation of phenotypic correlations using summary association statistics

Ting Li[1], *Zheng Ning*[2], ***Xia Shen***[3]

[1]*Sun Yat-sen University, Guangzhou, China,* [2]*Karolinska Institutet, Stockholm, Sweden,* [3]*University of Edinburgh, Edinburgh, United Kingdom.*

Estimating the phenotypic correlations between complex traits and diseases based on their genome-wide association summary statistics has been a useful technique in genetic epidemiology and statistical genetics inference. Two state-of-the-art strategies, Z-score correlation across null-effect SNPs and LD score regression intercept, were widely applied to estimate phenotypic correlations. Here, we propose an improved Z-score correlation strategy based on SNPs with low minor allele frequencies (MAFs), and show how this simple strategy can correct the bias generated by the current methods. Comparing to LDSC, the low-MAF estimator improves phenotypic correlation estimation thus is beneficial for methods and applications using phenotypic correlations inferred from summary association statistics.

T. Li: None. **Z. Ning:** None. **X. Shen:** None.

P17.056.C Prediction of eye, hair and skin color in admixed populations of Latin America

Sagnik Palmal[1], *Kaustubh Adhikari*[2,3], *Rolando González-José*[4], *Lavinia Schüler-Faccini*[5], *Maria-Cátira Bortolini*[5], *Victor Acuña-Alonzo*[6], *Samuel Canizales-Quinteros*[7], *Carla Gallo*[8], *Giovanni*

Poletti[8], Gabriel Bedoya[9], Francisco Rothhammer[10,11], Pierre Faux[1], Andres Ruiz-Linares[1,12,13]

[1]UMR 7268 ADES, CNRS, Aix-Marseille Université, EFS, Faculté de médecine Timone, Marseille, 13005, France, [2]The Open University, Milton Keynes, MK7 6AA, United Kingdom, [3]University College London, London, WC1E 6BT, United Kingdom, [4]Instituto Patagonico de Ciencias Sociales y Humanas, Centro Nacional Patagonico, CONICET, Puerto Madryn, U9129ACD, Argentina, [5]Departamento de Genética, Universidade Federal do Rio Grande do Sul, Porto Alegre, 90040-060, Brazil, [6]National Institute of Anthropology and History, Mexico City, 6600, Mexico, [7]UNAM-Instituto Nacional de Medicina Genómica, Mexico City, 4510, Mexico, [8]Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima,31, Peru, [9]GENMOL (Genética Molecular), Universidad de Antioquia, Medellín, 5001000, Colombia, [10]Instituto de Alta Investigación, Universidad de Tarapaca, Arica, 1000000, Chile, [11]Programa de Genetica Humana, ICBM, Faculdad de Medicina, Universidad de Chile, Santiago, 1000000, Chile, [12]Department of Genetics, Evolution and Environment, and UCL Genetics Institute, University College London, London, WC1E 6BT, United Kingdom, [13]Ministry of Education Key Laboratory of Contemporary Anthropology and Collaborative Innovation Center of Genetics and Development, School of Life Sciences and Human Phenome Institute, Fudan University, Yangpu District, Shanghai, China.

Abstract body

There is increasing interest in the use of genetic information for predicting physical appearance traits particularly in forensics and palaeoanthropology. We report an evaluation of prediction accuracy for eye, hair and skin pigmentation based on genomic and phenotypic data for over 6,500 admixed Latin Americans (the CANDELA dataset).

We examined the impact on prediction accuracy of three main factors: (i) The methods of prediction, including classical statistical methods and machine learning approaches, (ii) The inclusion of non-genetic predictors, continental genetic ancestry and pigmentation SNPs in the prediction models, and (iii) Compared two sets of pigmentation SNPs: the commonly-used HIrisPlex-S set (developed mainly in Europeans) and novel SNP sets that we have defined based on association results in the CANDELA samples.

We find that Random Forest or regression are globally the best performing methods. Although continental genetic ancestry has substantial power for prediction of pigmentation in Latin Americans, the inclusion of pigmentation SNPs increases prediction accuracy considerably, particularly for skin color. For hair and eye color, HIrisPlex-S has a similar performance to the CANDELA-specific sets.

This study shows that phenotypes with more variability in a specific ancestry population tend to show better prediction accuracy - eye color and hair color exhibit more variability in the european population but not the same for skin color. Thus investigating in a non-European population for skin color, allowed us to achieve better predictive power than HIrisPlex-S.

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P17.057.D Total genetic contribution assessment across the human genome

***Ting Li[1]**, Ning Zheng[1,2], Zhijian Yang[1], Ranran Zhai[1], Chenqing Zheng[1], Wenzheng Xu[1], Yipeng Wang[1], Yiwen Chen[1,3], Kejun Ying[4,5], Xia Shen[1,2,6]*

[1]Sun Yat-sen University, Guangzhou, China, [2]Karolinska Institutet, Stockholm, Sweden, [3]Uppsala University, Uppsala, Sweden, [4]Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA, [5]Harvard University, Boston, MA, USA, [6]University of Edinburgh, Edinburgh, United Kingdom.

Quantifying the overall magnitude of every single locus' genetic effect on the widely measured human phenome is of great challenge. We introduce a unified modelling technique that can consistently provide a total genetic contribution assessment (TGCA) of a gene or genetic variant without thresholding genetic association signals. Genome-wide TGCA in five UK Biobank phenotype domains highlighted the *HLA* locus for medical conditions, the bone mineral density locus *WNT16* for physical measures, and the skin tanning locus *MC1R* and smoking behaviour locus *CHRNA3* for lifestyle, etc. Tissue-specificity investigation revealed several tissues associated with total genetic contributions, including the brain tissues for mental health. Such associations were driven by tissue-specific gene expressions, which share genetic basis with the total genetic contributions. TGCA can provide a genome-wide atlas for the overall genetic contributions in each particular domain of human complex traits.

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P17.058.A Development of fine-map based polygenic risk scores: an analysis of genetic prediction models for height and LDL cholesterol in UK Biobank

***Carlo Maj[1]**, Christian Staerk[2], Oleg Borisov[1], Peter Krawitz[1], Andreas Mayr[2]*

[1]Institute for Genomic Statistics and Bioinformatics (IGSB), Faculty of Medicine, University of Bonn, Germany, Bonn, Germany, Bonn, Germany, [2]Department of Medical Biometry, Informatics and Epidemiology, Faculty of Medicine, University of Bonn, Germany, Bonn, Germany.

Polygenic risk scores (PRS) represent a quantification of the individual genetic predisposition with respect to a given phenotype. In the last few years several approaches to compute PRS have been implemented, the major difference across the methods is the modeling of the linkage disequilibrium (LD). The standard approach is to use clumping (to filter for independent variants) while other methods are based on the shrinkage of effect estimates according to reference LD panels via Bayesian methods (e.g., LDpred, PRSCS) or penalized regression (e.g., Lassosum). In the present work we applied different statistical learning methods for variable selection in high-dimensional linear regression models (including the Adaptive Subspace method, AdaSUb, and statistical boosting with probing, SBP) to fine-map the signal in significant genomic loci. The analysis has been performed on UK Biobank imputed genotype counts (273,440 samples for training and 135,444 samples for test, filtered for British ancestry). Two quantitative heritable and polygenic quantitative traits were considered, namely height and LDL cholesterol levels. Preliminary results show that both AdaSub with the extended Bayesian information criterion for variant selection (EBIC) and SBP reach a better accuracy than univariable clump-based PRS (cPRS) while including a small number of variants (height RMSE = 8.928;8.755;8.951; variants in the final model = 292;7668;26184, LDL RMSE = 0.827;0.823;0.841; variants in the final model =105;761;1660, values are for EBIC1, SBP and cPRS respectively). The proposed sparse fine-mapped models enhance the biological interpretability and may potentially reduce the

confounding due to LD-heterogeneity in different cohorts when comparing PRS across ethnicities.

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P17.059.B Prediction of the splicing effects of SNVs affecting the first nucleotide G of an exon

Atefeh Joudaki, *Jun-ichi Takeda, Akio Masuda, Kinji Ohno*

Neurogenetics, Nagoya University Graduate School of Medicine, Nagoya, Japan.

Various tools have been developed to predict the splicing effects of SNVs (Abramowicz and Gos. J Appl Genet. 2018). However, the splicing effects of SNVs at the first nucleotide G of exons are complicated. It is evident that various factors significantly contribute to the splicing pattern of SNVs at the first nucleotide G of an exon, including the AG-dependence of the 3′ splice site (ss) and interaction with U2AF35 (Gao *et al*. Nucleic Acids Res. 2011; Yoshida *et al*. Nat Commun. 2020).In our research, random forest (RF) models were generated by 66 splicing-affecting SNVs in the Human Gene Mutation Database (HGMD) and 83 neutral SNVs in the dbSNP database [minor allelic frequency (MAF) ≥ 0.01] using 108 features including exon length, the number of pyrimidines in the polypyrimidine tract (PPT), the position of predicted branch-point sequence (BPS), the sequence of predicted BPS, individual nucleotides at intron -3 and exon +1, the strength of splicing signals at the 5′ and 3′ ss's, and motifs of RNA-binding proteins to name a few. Using ten-fold cross-validation, our models showed that the area under the receiver operating characteristic curve (AUROC), and the area under the precision-recall curve (AUPR) were on average 0.943 and 0.954, respectively.

A. Joudaki: None. **J. Takeda:** None. **A. Masuda:** None. **K. Ohno:** None.

P17.060.C The possibilities of artificial intelligence for the assessment of genetic predisposition factors and the diagnosis of colorectal cancer

Igor Ugarov[1], *Vyacheslav Chernykh*[2,1], *Inna Sharkova*[1,3], *Nikolay Ivanov*[1], *Vladimir Maslennikov*[1], *Dmitriy Ostapenko*[1]

[1]*xGen Cybernetics, Moscow, Russian Federation,* [2]*Federal state scientific budgetary Institution «Research Centre for Medical Genetics», Moscow, Russian Federation,* [3]*Federal state scientific budgetary Institution «Research Centre for Medical Genetics», Moscow, Russian Federation.*

Colorectal cancer (or CRC) is a malignant multifactorial (polygenic) neoplasm that occupies a leading position among all oncological diseases in terms of prevalence in Russia and the world. Objective: to develop a genetic panel and identify molecular patterns in the results of genetic testing for diagnosis of colorectal cancer.

Materials and methods: For a set of genes, the analysis of genomic databases and refereed foreign scientific articles on the following keywords was performed: "colorectal cancer", "mutation", "miRNA", "CNV", "single-nucleotide polymorphism". To evaluate the performance of the model, we used data from The Cancer Genome Atlas (TCGA, https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga). Based on the data, two groups were formed: patients with colorectal cancer (n = 233) and a cohort of patients with other nosologies acting as a control group (n = 6513).

Results: A method has been developed that allows us to identify screening patterns of multifactorial conditions, taking into account the polygenic nature of diseases and the uncertainty of the pathogenic effect of individual genetic variants. Epigenetic patterns included 56 microRNAs. The Se and Sp were 98% and 62%, respectively. Methylation patterns included changes in methylation in 72 genes. The Se and Sp was 34% and 99%. The protein patterns included 38 proteins. The Se and Sp of this model was 68% and 65%. The developed approach can also be used to identify genotypes and epigenotypes characteristic of the development of other multifactorial conditions and polygenic diseases.

I. Ugarov: None. **V. Chernykh:** None. **I. Sharkova:** None. **N. Ivanov:** None. **V. Maslennikov:** None. **D. Ostapenko:** None.

P17.061.D Polygenic risk score strategies for transcriptome-wide association analysis in prostate cancer risk

Nicholas B. Larson, *Shannon K. McDonnell, Zachary Fogarty*

Mayo Clinic, Rochester, MN, USA.

Statistical methods for transcriptome-wide association studies (TWAS) are predicated on co-localization of single-SNP associations from two distinct datasets: (1) eQTLs from gene expression studies and (2) SNP-phenotype associations from GWAS. A practical limitation of TWAS is the emphasis on cis-eQTL effects on expression due to limited sample sizes of eQTL datasets, which restricts discovery power for trait-related genes whose expression may be influenced by aggregate trans-acting effects. An alternative TWAS approach is to translate the genome-wide associations from GWAS to eQTL datasets via polygenic risk scores (PRSs). Herein, we investigated associations between gene expression and a PRS for prostate cancer (PRCA), a highly heritable and polygenic disease. We applied Lassosum to generate a weighted PRCA PRS with 26,539 total SNPs based on PRCA risk GWAS summary statistics from the PRACTICAL Consortium. Using a large normal prostate tissue eQTL dataset (N = 471) with RNA-Seq and genotyping data, we tested the Kendall's tau rank correlation between normalized gene expression and the PRCA PRS across the full transcriptome. To accommodate more flexibility, we explored variance-component testing via SKAT using PRS-weighted linear kernels. We also compared these PRS-based results with TWAS findings from FUSION applied to the same datasets. No individual PRS-expression correlations were statistically significant (FDR < 0.05), although KEGG and REACTOME GSEA results revealed significant associations with relevant gene-sets, including GnRH and phosphatidylinositol signaling pathways. SKAT-based testing resulted in 11 significant genes (FDR < 0.05), notably none of which were identified via FUSION. Future efforts will further evaluate statistical considerations of complementary polygenic approaches to traditional TWAS.

N.B. Larson: None. **S.K. McDonnell:** None. **Z. Fogarty:** None.

P17.062.A Longitudinal MicroRNA Signature of Conversion to Psychosis

Anton Iftimovici[1,2], *Qin He*[2], *Chuan Jiao*[2], *the ICAAR study group, Edouard Duchesnay*[1], *Marie-Odile Krebs*[2], *Oussama Kebir*[2], *Boris Chaumette*[2]

[1]*NeuroSpin, CEA, Paris, France,* [2]*Université de Paris, Institute of Psychiatry and Neuroscience of Paris (IPNP), INSERM U1266, GDR 3557-Institut de Psychiatrie, Paris, France, Paris, France.*

Introduction: Epigenetics are key to the gene x environment interactions leading to psychosis. We postulated that longitudinal changes in microRNAs could be a signature of psychotic transition.

Material and methods: Next-generation high-throughput sequencing of microRNAs was done at two time-points in 81 at-risk subjects (of whom 35 transited). MicroRNA variation across time (Δmirna) was computed as the difference between baseline and follow-up microRNA measures, divided by the subject's follow-up time. Three methods were combined to find Δmirna associated with psychosis: 1) a differential expression analysis of Δmirna after stringently filtering microRNAs sequenced in all samples at all times (77 among 2479 detected in total); 2) a supervised algorithm with a ridge classifier applied to all 2479 Δmirna to identify the variations in expression most relevant for prediction; 3) a differential network analysis applied to all Δmirna to identify interactions specific to converters or non-converters.

Results: miR-150-5p variation across time differed significantly between groups, after FWER correction. The machine-learning strategy predicted psychosis with an area-under-the-curve of 66 % (non-parametric $p = 0.009$), and 207 Δmirna were confidently leveraged for prediction, with bootstrapped 95% confidence intervals excluding zero. There were 276 Δmirna with interactions specific to either converters or non-converters.

Discussion: Combining three different strategies, we reduced the risk of methodological biases associated with any single method. Crossing machine-learning and network analyses, we identified 25 microRNAs. Their 438 gene targets were enriched in schizophrenia GWAS genes and synaptic processes. All analyses highlighted miR-150-5p, a microRNA involved in cognition.

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P17.063.B Phenotype-Tissue Expression and Exploration (PTEE) facilitates RNA-seq-based Mendelian disease diagnosis

Akhil Velluva[1,2], *Maximillian Radtke*[3], *Susanne Horn*[2], *Konrad Pltazer*[3], *Erind Gjermeni*[4], *Johannes R. Lemke*[3], *Torsten Schöneberg*[2], *Janet Kelso*[1], *Rami Abou Jamra*[3], *Diana Le Duc*[1,3]

[1]*Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany,* [2]*Rudolf Schönheimer Institute of Biochemistry, Medical Faculty, University of Leipzig, Leipzig, Germany,* [3]*Institute of Human Genetics, University Medical Center, Leipzig, Germany,* [4]*Department of Electrophysiology, Heart Center Leipzig at University of Leipzig, Leipzig, Germany.*

RNA-seq has gained more visibility by holding the promise to improve the diagnostic yield in unresolved cases of rare Mendelian diseases. However, RNA tissue specificity complicates the landscape, especially when given a phenotype, the tissue of interest (e.g. brain) cannot be accessed. Based on data from Genotype-Tissue Expression Project we developed a web tool - PTEE https://bioinf.eva.mpg.de/shiny/PTEE/, which allows clinicians to decide on the most appropriate tissue to inquire for a patient's phenotype and a tissue of interest. As a sanity check we prove that the most suitable accessible tissue to investigate heart arrhythmias is skeletal muscle. We show that for NDD, skin has the best correlation to the central nervous system (CNS) ($r = 0.99$) and shares 75% of the transcripts expressed in the brain. While the correlation of blood and brain expression for NDD genes is acceptable ($r = 0.87$), only 47% of the brain transcripts are shared. Interestingly, 19 of the NDD genes are not expressed in the CNS, but are expressed in at least one of the accessible tissues. These genes cluster in GO terms related to cell cycle and cell division ($p < 0.01$), suggesting they are involved in developmental processes. Thus, for RNA-seq there is no single tissue, which assures the discovery of the causative/candidate gene for a phenotype. Clinicians can benefit from our tool to make an informed decision on which accessible tissue is most suitable for RNA-seq and increase the diagnostic chances.

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P17.064.C De novo variants within constrained coding regions are a major source of pathogenicity for rare diseases

Hywel J. Williams[1], *Chris Odhams*[2], *Genomics England*[2]

[1]*Cardiff University, Cardiff, United Kingdom,* [2]*Genomics England, London, United Kingdom.*

De novo (Dn) variants affecting protein-coding DNA are a well-established cause of Rare Diseases (RDs) in patients with a neurological/neurodevelopmental (NND) phenotype but their evaluation across the full-spectrum of clinical RD phenotypes has not been performed at scale. Constrained coding regions (CCRs) are specific segments of coding DNA that are devoid of functional variants in healthy individuals but enriched for pathogenic mutations.

We have sought to evaluate the utility of incorporating CCRs into the diagnostic filtering cascade of RD patients that have undergone genomic sequencing and, specifically, determined the contribution Dns play in improving diagnostic rate.

We have used data from 6144 trios that have undergone diagnostic evaluation as part of the Genomics England 100,000 Genomes Project, including 2715 trios analysed with an advanced Dn identification pipeline.

We show in the full dataset, 12% of patients classed as solved have a variant within a CCR while, in the subset of patients that have undergone Dn evaluation, this rises to 18%. Enrichment analysis of the individual phenotype classifications shows highly significant but contrasting results. For example, pathogenic Dn variants intersecting a CCR were overrepresented in the NND group ($p = 8.13 \times 10^{-06}$) but underrepresented in the Ophthalmological group ($p = 2.47 \times 10^{-07}$).

Our results demonstrate the potential clinical utility of performing bespoke Dn analyses of RD patients and for incorporating CCR information into the filtering cascade. However, questions remain about why different phenotypic classifications differ so markedly in their enrichment of pathogenic Dn variants in CCRs.

H.J. Williams: None. **C. Odhams:** None. **G. England:** None.

P17.065.D Rare diseases have many faces: The road to diagnostic success

Simona Denise Frederiksen, *Vladimir Avramovic, Maja Tarailo-Graovac*

University of Calgary, Calgary, AB, Canada.

Introduction: In the Silent Genomes project, we seek to improve the diagnostic success for Indigenous children with genetic diseases. One obstacle we are facing is that many patients remain undiagnosed even after whole-genome sequencing. We hypothesize that the genetic diseases in some of these patients stem from more complex genetic scenarios than single gene defects. The aim of this study is to better understand the rare disease spectrum.

Materials and Methods: We identified rare diseases using Orphadata (orientdb version) and categorized them into 3 categories based on prevalence, borderline-common (1-9 cases per 10,000), rare (1-9 cases per 100,000 and 1,000,000) and ultra-rare (less than 1 case per 1,000,000). Each of these categories were dissected focusing on variables such as associated phenotypes and genes.

Results: A total of 145 borderline-common, 412 rare and 2967 ultra-rare diseases constituted the rare disease spectrum. Differences between the disease categories were observed for multiple variables including inheritance mode and associated phenotypes and genes. We found that when diagnosed with a borderline-common disease, patients are more likely to experience phenotypic variability, which may complicate data analysis methods focused on single gene defects. In agreement, our results showed a higher proportion of borderline-common rare diseases caused by DNA sequence changes in multiple genes or involvement of multiple factors compared with the other categories.

Conclusions: These findings will help triage 'difficult-to-diagnose' rare disease patients to better defined sub-categories and devise appropriate statistical approaches to determine underlying disease causes (e.g. search for more than one damaged gene).

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P17.066.A The Regulatory Mendelian Mutation (ReMM) score for GRCh38

Lusiné Nazaretyan[1,2], *Max Schubach*[1,2], *Martin Kircher*[1,2]

[1]*Charité – Universitätsmedizin Berlin, Berlin, Germany,* [2]*Berlin Institute of Health, Berlin, Germany.*

Despite catalogs of more than 7,000 known Mendelian disorders, very few non-coding variants have been identified as disease causative so far. While there is a consensus that regulatory mutations play an important role, computational tools that support their identification are still lacking. Here, we rebuild the ReMM score (Smedley D. et al. AJHG 2016) for prioritizing non-coding mutations in the GRCh38 genome assembly. We contrast a curated set of 406 regulatory variants causative for Mendelian disorders and millions of human-derived sequence alterations (as proxy for non-pathogenic variation) in the human genome. We use a set of 26 genomic features combining epigenetic profiles, species conservation and density of disease and population variants to train the hyper-ensemble random forest model hyperSMURF (Petrini A. et al. Gigascience 2020). Our workflow from acquiring the raw data up to calculating model scores is based on Snakemake (Köster and Rahmann, 2012). This improves reproducibility and facilitates adaption of the model for future genome releases and inclusion of new features. We achieve an average precision of 0.57 on our data, which compares favorably to the original ReMM version of the GRCh37 build (0.50). We observe moderate correlation of scores (0.72) between genome builds, which we ascribe to the changes in the feature sets as well as adjustments in feature importance. Our work offers a reliable tool for scoring pathogenicity of human regulatory variants and will facilitate further research in the field of prioritizing variants in the non-coding genome.

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P17.067.B TGG-Viewer: Web-based Interactive Genome-wide Visualization of RNAseq Data

Ben Weisburd

Broad Institute, Cambridge, MA, USA.

Visualizations of RNAseq splice junctions and expression levels can help users manually review candidate disease-causing loci and identify patterns and technical error modes. To address limitations of existing visualization tools, we created an online viewer based on IGV.js that enables sashimi-like visualizations of splice junctions and coverage that are interactive, genome-wide, and support any zoom level. The viewer is freely available at http://tgg-viewer.broadinstitute.org and includes the features listed below.

- splice junction and coverage tracks for 1 or more samples can be displayed along-side gene tracks, BAM/CRAM data, SNP or CNV callsets, normalized coverage tracks such as those generated by gCNV, and other kinds of genomic feature tracks.
- new reference tracks are provided for GTEx muscle, blood and fibroblast tissues to summarize all splice junctions in all GTEx samples from these tissues, and allow comparison to rare disease samples.
- splice junctions can be filtered or shown in different colors based on read support, strand, whether the junction is novel, and other criteria.
- to visualize their own data, users don't need to upload it to a tgg-viewer server or create a user account. Instead, they upload their data to their own Google bucket, log in to tgg-viewer with their Google account.
- to enable efficient visualization at any zoom level, and to allow arbitrary numbers of samples to be combined into a single input file, we use BED as the underlying format instead of BAM/CRAM.

B. Weisburd: None.

P17.069.D Comparison of methods of genotyping short tandem repeats (STRs) from whole genome sequences

John W. Oketch, *Louise V. Wain, Edward J. Hollox*

University of Leicester, Leicester, United Kingdom.

Short tandem repeats (STRs) are repeated regions of genomes that consist of a simple sequence motif 2-6 bp tandemly-repeated multiple times. Variations in STRs cause over 40 monogenic disorders including Huntington disease. In addition, STRs are known to affect gene expression levels and have been implicated in complex diseases for example autism. However, STRs remain understudied owing to difficulties and precision of genotyping STRs at a large scale. Recently, new tools to genotype STRs from short read genome sequences have become available, including STRetch, GangSTR and HipSTR. To assess their applicability to large cohorts of genome sequences sequenced at ~30x, this pilot study compared the tools using seven gold standard human genome sequences from the Genome in a Bottle consortium, including two parent-child trios. These individuals have also been sequenced using long read technologies.First, STR calls were made at initial genome coverage of 100x or 300x using the recommended tool-specific set of reference STRs. Secondly, we downsampled the genomes to 30x-coverage and repeated our analysis.The number of genotypes calls made by Stretch varied by coverage from as low as 2310 (at 30x coverage) to ~16,000 (300x). HipSTR and GangSTR reported consistent number of calls ~1.4x10^6 at each coverage with about 70% overlaps between the calls. About 92% of calls made by HipSTR and 97% by GangSTR followed Mendelian inheritance patterns indicating high robustness in genotype calls.We aim to use the selected tools on large case-control cohorts, by investigating the genomewide association of STRs with complex respiratory disease.

J.W. Oketch: None. **L.V. Wain:** None. **E.J. Hollox:** None.

P17.070.A scMuffin: an R package for resolving solid tumor heterogeneity from single-cell data

Noemi Di Nanni, Cinzia Cocola, Valentina Nale, Eleonora Piscitelli, Alice Chiodi, Ingrid Cifola, Ileana Zucchi, Rolland Reinbold, Luciano Milanesi, Alessandra Mezzelani, Paride Pelucchi, ***Ettore Mosca***

Institute of Biomedical Technologies, National Research Council, Segrate (Milan), Italy.

Introduction: ingle cell (SC) analysis is crucial to study the complex cellular heterogeneity of solid tumors, which is one of the main obstacles for the development of effective cancer treatments. Such tumors typically contain a mixture of cells with aberrant genomic and expression profiles affecting specific sub-populations that have a pivotal role in cancer progression, whose identification eludes bulk approaches. We present a MUlti-Features INtegrative approach for SC data analysis (scMuffin) that characterizes cell identity on the basis of multiple and complementary criteria.

Materials and Methods: Cell markers sources: CSEA, PanglaoDB. Pathways sources: NCBI Biosystems, MSigDB. Gene set expression is assessed by a fast algorithm that uses comparable control-gene sets. CNVs are estimated using adjacent genes. Lineage analysis is computed by Monocle; multipotency is assessed by LandScent. The association between the various features and cell clusters is assessed by chi-squared and enrichment-based approaches.

Results: scMuffin provides functions to calculate a series of qualitative and quantitative scores, such as: expression of markers for normal and tumor conditions, pathway activity, cell hierarchy, multipotency state, copy number variations and cell cycle state. Cell-level scores are used for cell cluster annotation and combined to obtain alternative cell clusters. scMuffin integrates any type of cell- or cluster-associated data, and can be used for single-cell multi-omics analyses (e.g. mutations, gene expression). As a proof-of-principle, we studied a public dataset of human gliomas.

Conclusions: scMuffin combines several tools to shed light on the identity of tumors cells and spot subtle cell types. Funding: MIUR INTEROMICS PB05.

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P17.071.B Benchmarking deep learning splice prediction tools using functional splice assays

Tabea V. Riepe[1], *Mubeen Khan*[2], *Susanne Roosing*[1], *Frans P. M. Cremers*[1], *Peter A. C. 't Hoen*[1]

[1]*Radboud University Medical Center, Nijmegen, Netherlands,* [2]*University Medical Center Groningen, Groningen, Netherlands.*

Introduction: Hereditary disorders are frequently caused by genetic variants that affect pre-mRNA splicing. Whilst genetic variants in the canonical splice motifs are almost always disrupting splicing, the pathogenicity of variants in the non-canonical splice sites (NCSS) and deep intronic (DI) regions are difficult to predict. Multiple splice prediction tools have been developed for this purpose, with the latest tools employing deep learning algorithms. We benchmarked established and deep learning splice prediction tools on gold standard sets of variants in the *ABCA4* and *MYBPC3* genes associated with Stargardt disease and cardiomyopathy, respectively, with functional assessment splice assays.

Methods: Twelve freely available splice prediction tools that either use deep learning or are widely applied in routine diagnostics were applied to 71 *ABCA4* NCSS, 81 *ABCA4* DI and 61 *MYBPC3* NCSS variants. Receiver operator curves, accuracy, sensitivity, specificity, positive predictive value, negative predictive value and Mathew's correlation coefficient were used to evaluate the performance of each tool on the different datasets.

Results: Based on the receiver operator curves the five best performing tools for each dataset were determined. For *ABCA4* NCSS variants those tools were SpliceAI, DSSP, S-CAP, Spidex and MaxEntScan. The best performing tools for the *ABCA4* DI variants were SpliceAI, SpliceRover, MaxEntScan, NNSplice and Alamut 3/4. For *MYBPC3* NCSS variants, SpliceSiteFinder-like, Alamut 3/4 MaxEntScan, NNSplice and GeneSplicer achieved the highest AUC.

Conclusions: The choice of splice prediction tool depends on the dataset. SpliceAI, the Alamut 3/4 consensus approach, NNSPLICE and MaxEntScan perform well on all three datasets.

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P17.072.C SVInterpreter: a web-based tool for structural variants inspection and identification of possible disease-causing candidate genes

Joana Fino[1], *Bárbara Marques*[1], *Zirui Dong*[2], *Dezsö David*[1]

[1]*National Health Institute Doutor Ricardo Jorge, Lisbon, Portugal,* [2]*Chinese University of Hong Kong, Hong Kong, Hong Kong.*

Introduction: With the advent of genomic sequencing, the identification of structural variants (SVs), including copy number variants, is no longer a challenge: recent studies showed that is possible to detect up to 9 K SVs per individual. Contrarily, the annotation of the genome is incomplete, and the data is scattered along different databases, making SV manual evaluation almost impossible. Also, the available tools are limited in their scope. Thus, to address the need of a comprehensive application to assist evaluation of clinical outcome of SVs, we developed Structural Variant Interpreter (SVInterpreter).

Methods: SVInterpreter is a free Python-CGI developed Web application able to analyze SVs using Topologically Associated Domains as genome units, within which genome browsers data, medically actionable genes, virtual gene panels and HPO similarity results, among other, is retrieved.

Results: First, we reanalysed 222 published SVs, of which about 58% were previously classified as VUS. SVInterpreter corroborated the previous classification in about 84% of the SVs. In about 5% of the SVs, SVInterpreter gave indication of possible position effect, through phenotype similarity, disrupted chromatin loops or genome wide association studies. Then, we show the applicability of SVInterpreter in the clinical context, by inspecting 20 cases analysed by chromosomal microarray or genome sequencing.

Conclusions: To our knowledge, SVInterpreter is the most comprehensive TAD based tool to assist prediction of clinical outcome of SVs. Based on gathered information, identification of possible disease-causing candidate genes and SVs is easily achievable. SVInterpreter is available at http://dgrctools-insa.min-saude.pt/cgi-bin/SVInterpreter.py

J. Fino: None. **B. Marques:** None. **Z. Dong:** None. **D. David:** None.

P17.073.D iROAR: a computational algorithm for multiplex PCR bias detection and correction in TCR repertoires datasets

Anastasia O. Smirnova[1,2], *Yuri B. Lebedev*[2], *Ilgar Z. Mamedov*[2,3], *Alexander Y. Komkov*[2,3]

[1]Skolkovo Institute of Science and Technology, Moscow, Russian Federation, [2]Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russian Federation, [3]Dmitry Rogachev National Medical and Research Center of Pediatric Hematology, Moscow, Russian Federation.

Introduction: High-throughput sequencing of T-cell receptor (TCR) repertoires is widely used to investigate adaptive immunity genomics. Multiplex PCR is most reliable and cost-effective method for TCR library preparation. The main unsolved challenge in such methods is enormous amplification bias which significantly complicates the downstream analysis. Here we describe a first fully computational algorithm for PCR bias removing in TCR repertoires called iROAR.

Material and methods: 15 low biased 5′-RACE based TCR repertoires and 30 highly biased multiplex PCR based TCR repertoires downloaded from https://www.ncbi.nlm.nih.gov/sra were used for this study. Based on statistical features of nonfunctional TCR rearrangements in low biased TCR repertoires we formulated the Over Amplification Rate (OAR) measure as a ratio of observed and expected frequency of a V and a J gene among nonfunctional rearrangements. OAR is equal to 1 in the absence of PCR bias and deviates from 1 if distinct V or J gene is over- or under-represented in library.

Results: Using OAR concept, we developed an original algorithm to reduce PCR bias. The idea is based on iterative clone count compensation by dividing of each clone count by normalization coefficient - OAR(Vi)*OAR(Ji) - for each particular V-J combination. Efficacy of iROAR was successfully validated on 5′-RACE TCR dataset with *in silico* introduced bias, and by comparison of two methods: 5′-RACE and two-side multiplex.

Conclusion: The developed algorithm was implemented as command-line software which is openly available for research use at https://github.com/smiranast/iROAR. This work was supported by RSF grant 20-75-10091.

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P17.074.A KidneyNetwork: Using kidney-derived gene expression data to predict and prioritize novel genes involved in kidney disease

Floranne Boulogne[1], *Laura R. Claus*[2], *Henry Wiersma*[1], *Roy Oelen*[1], *Floor Schukking*[1], *Niek de Klein*[1], *Shuang Li*[1], *Harm-Jan Westra*[1], *Bert van der Zwaag*[2], *Franka van Reekum*[2], *Dana Sierks*[3], *Ria Schönauer*[3], *Jan Halbritter*[3], *Nine V. A. M. Knoers*[1], *Genomics England Research Consortium, Patrick Deelen*[1], *Lude Franke*[1], *Albertien M. van Eerde*[2]

[1]UMC Groningen, Groningen, Netherlands, [2]UMC Utrecht, Utrecht, Netherlands, [3]University of Leipzig Medical Center, Leipzig, Germany.

Introduction: Genetic testing in patients with suspected hereditary kidney disease may not reveal the genetic cause for the disorder as potentially pathogenic variants can reside in genes that are not known to be involved in kidney disease. To help identify these genes, we have developed KidneyNetwork, in which tissue-specific expression is utilized to predict kidney-specific gene functions.

Material and Methods: KidneyNetwork is a co-expression network built upon a combination of 878 kidney RNA-sequencing samples and a multi-tissue dataset of 31,499 samples. It uses expression patterns to predict which genes have a kidney-related function and which phenotypes might result from mutations in these genes. As proof of principle, we applied KidneyNetwork to prioritize rare variants in exome-sequencing data from 13 kidney disease patients without a genetic diagnosis.

Results: KidneyNetwork can accurately predict kidney-specific gene functions and (kidney disease) phenotypes for disease-associated genes and applying it to exome-sequencing data of kidney disease patients allowed us to identify a promising candidate gene for kidney and liver cysts: ALG6.

Conclusion: We present KidneyNetwork, a kidney-specific co-expression network that accurately predicts which genes have kidney-specific functions and can result in kidney disease. We show the added value of KidneyNetwork by applying it to kidney disease patients without a molecular diagnosis. KidneyNetwork can be applied to clinically unsolved kidney disease cases, but it can also be used by researchers to gain insight into individual genes in order to better understand kidney physiology and pathophysiology. Grant references: This research was supported by the Dutch Kidney Foundation (18OKG19).

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P17.075.B Deciphering the role of trasposable elements in CD4$^+$tumor-infiltrating lymphocytes at single cell resolution

Valeria Ranzani[1], *Benedetto Polimeni*[2,1], *Annarita Putignano*[1], *Samuele Notarbartolo*[1], *Valeria Bevilacqua*[1], *Serena Curti*[1], *Sergio Abrignani*[1,3], *Federica Marasca*[1], *Beatrice Bodega*[1]

[1]Istituto Nazionale Genetica Molecolare, Milano, Italy, [2]University of Milano-Bicocca, Medicina Traslazionale e Molecolare (DIMET), Milano, Italy, [3]University of Milan, Department of Clinical Sciences and Community Health, Milano, Italy.

Many TE families have a role in the regulation of the epigenome, 3D genome organization and the transcriptional output of the cell. While the roles of TEs in embryogenesis and development is already documented, their contribution to adult cell commitment and differentiation is still poorly investigated. Hence, we sought to decipher the role of TEs in regulating tumour-infiltrating lymphocytes (TILs) plasticity and their impact in the heterogeneity of T cell subsets in the tumour microenvironment. Preliminary results on RNA-FISH showed that TE-derived RNAs are more enriched in TILs compared to adjacent normal tissue. Here, we apply a custom pipeline for the quantification of TE expression from 5′-end 10X Genomics scRNA-seq data of circulating, colorectal cancer (CRC) and normal tissue-infiltrating CD3$^+$ T cells. We unveiled a hidden heterogeneity within subpopulations of CD3$^+$ T cells, characterized by expression patterns of distinct TE families specific for circulating or infiltrating lymphocytes. We detected cell clusters enriched in either evolutionarily old or young TE families, supporting the inclusion of TEs at the 5′-end of genes or the autonomous expression of TEs. These results suggest that TEs contribute to the identity of T lymphocytes in response to the exposure to the tumour microenvironment. We will expand this analysis by discovering signatures of TEs in subpopulations of TILs and profiling the TCR clonotypes of the same cells.

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P17.076.C Treatabolome database: towards enhancing Rare Diseases' treatment visibility

*Carles Hernandez-Ferrer[1], Alberto Corvó[1], Leslie Matalonga[1], Rachel Thompson[2], Leigh Carmody[3], Davide Piscia[1], Alfons Macaya[4], Angela Lochmuller[5], Alex Manta[2], Bertrand Fontaine[6], Sabine Vicart[7], Jean-François Desaphy[8], Concetta Altamura[8], Karim Wahbi[9], Annachiara De Sandre-Giovannoli[10], Corinne Vigouroux[11], Birte Zurek[12], Carola Rheinard[13], David Gómez-Andrés[4], Katherine Schon[5], Laura Over[14], Norbert Brüggemann[14], Katja Lohmann[14], Matthew J. Jennings[5], Matthis Synofzik[15], Olaf Riess[13], Rabah Ben Yaou[6], Teresinha Evangelista[16], Thiloka Ratnaike[17], Virginie Bros-Facer[18], Gulcin Gumus[18], Rita Horvath[5], Patrick Chinnery[5], Holm Graessner[13], Peter Robinson[3], **Antonio Atalaia**[6], Hanns Lochmuller[2], Gisèle Bonne[19], Sergi Beltran[1]*

[1]CNAG-CRG, Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Barcelona, Spain, [2]Children's Hospital of Eastern Ontario Research Institute, Canada, Ottawa, ON, Canada, [3]The Jackson Laboratory For Genomic Medicine, Farmington, CT 06032, USA, Farmington, CT, USA, [4]Paediatric Neurology, Vall d'Hebron University Hospital and VHIR (Euro-NMD, ERN-RND), Barcelona, Spain, [5]Department of Clinical Neurosciences, University of Cambridge School of Clinical Medicine, Cambridge Biomedical Campus, Cambridge, United Kingdom, [6]Sorbonne Université - Inserm UMRS 974, Center of Research in Myology, Institut de Myologie, G.H. Pitié-Salpêtrière Paris, France, Paris, France, [7]Sorbonne Université, INSERM, Assistance Publique Hôpitaux de Paris, Centre de Recherche en Myologie-UMR 974, Reference center in neuro-muscular channelopathies, Institute of Myology, Hôpital Universitaire Pitié-Salpêtrière, Paris, France, [8]Department of Biomedical Sciences and Human Oncology, School of Medicine, University of Bari Aldo Moro, Bari, Italy, [9]APHP, Cochin Hospital, Cardiology Department, FILNEMUS, Centre de Référence de Pathologie Neuromusculaire Nord/Est/Ile de France, Université de Paris, Paris, France, [10]AP-HM, Department of Medical Genetics, and CRB-TAC (CRB AP-HM), Children's Hospital La Timone, Marseille, France, [11]AP-HP Saint-Antoine Hospital, Reference Centre of Rare Diseases of Insulin Secretion and Insulin Sensitivity (PRISIS), Departments of Molecular Biology and Genetics and of Endocrinology, 75012, Paris, France, [12]Institute of Medical Genetics and Applied Genomics, University of Tuebingen, Germany, Tubingen, Germany, [13]Institute of Medical Genetics and Applied Genomics, University of Tuebingen, Tubingen, Germany, [14]Institute of Neurogenetics, University of Lübeck, Lubeck, Germany, [15]Department of Neurodegenerative Diseases, Hertie-Institute for Clinical Brain Research and Center of Neurology, University of Tübingen, Tubingen, Germany, [16]Unité de Morphologie Neuromusculaire, Institut de Myologie, GHU Pitié-Salpêtrière; Sorbonne Université, AP-HP, INSERM, Centre de référence Des Maladies Neuromusculaires Nord/Est, Ile de France, Paris, France, [17]Department of Paediatrics, Cambridge University Hospitals NHS Foundation Trust; Department of Clinical Neurosciences, University of Cambridge, Cambridge, United Kingdom, [18]EURORDIS – Rare Diseases Europe, Paris, France, [19]Sorbonne Université - Inserm UMRS 974, Center of Research in Myology, Institut de Myologie, G.H. Pitié-Salpêtrière, Paris, France.

Introduction: Although next-generation sequencing (NGS) has drastically improved diagnosis for patients with rare diseases (RDs), access to knowledge of effective treatments is still sparse and often unclear. The large number of RDs (>7,000 estimated) and their genetic heterogeneity make the identification of existing treatments difficult for clinicians. Herein we report Treatabolome DB, a database of RD-specific treatments mapped at gene and variant level to allow easy identification of published therapies.

Materials and methods: A relational database was developed to collect variant-to-treatment mappings from systematic literature reviews (SLRs) produced by disease experts. To date, 8 SLRs have been completed on congenital myasthenic syndromes, laminopathies, muscular channelopathies, mitochondrial disorders (Leigh syndromes), hereditary peripheral neuropathies, genetic forms of Parkinson´s disease, and metabolic myopathies; additional participation from RD experts is welcomed.

Results: A data model based on the use of public ontologies and international recommendations was defined by the Treatabolome working group to enable system interoperability. The Treatabolome DB schema is based on data submission and allows discoverability of information from the SLRs. Treatabolome DB will be publicly accessible through programmatic interfaces and a web portal supporting queries of terms including diagnostic (ORDO, OMIM, and HPO), gene, variant, and treatment (ChEBI, UMLS or MeSH).

Conclusion: Treatabolome DB enables identification of existing treatments for RD patients at the time of diagnosis. Future developments include its connection with genomic analysis tools such as the RD-Connect GPAP. **Funding**: Solve-RD project (H2020 #779257), ERN for Neuromuscular Diseases (#870177) ERN for Rare Neurological Diseases (#739510).

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P17.077.D Exploring the causality of epidemiological relationships between Type 2 Diabetes and cancers using pathway-specific genetic instruments

Zhanna Balkhiyarova[1], *Anna Ulrich[1], Ayse Demirkan[1], Liudmila Zudina[1], Igor Pupko[1], Jared G. Maina[2], Philippe Froguel[2], Marika Kaakinen[1], Inga Prokopenko[1]*

[1]University of Surrey, Guilford, United Kingdom, [2]University of Lille, Lille, France.

Introduction: Epidemiological studies suggest that people with type 2 diabetes (T2D) are at risk for various cancers. T2D and postmenopausal breast (BrC), colorectal (CrC), prostate (PrC) and pancreatic (PanC) cancers share many risk factors, but potential biological links between them are incompletely understood. We aimed at investigating causality by pathophysiological process between these diseases using the Mendelian Randomisation (MR) approach.

Materials and Methods: We first used agglomerative hierarchical clustering to group T2D-associated and four cancers risk-contributing SNPs by biological pathways based on their effects on 45 metabolic-/inflammatory-/tumour-/obesity-related phenotypes. The cardiovascular cluster comprised 74 variants with effects on insulin secretion and cardiovascular risk. The hormonal-lipid cluster grouped 85 SNPs with shared effects on hormone and lipid levels. The glycaemic cluster highlighted 28 T2D SNPs with effects on glycaemia. We applied a two-sample MR framework to investigate the role of these three pathways in developing cancer. Effect estimates for the same or proxy variants ($r^2 > 0.8$) on T2D and cancers were obtained from the largest-to-date respective GWAS.

Results: The hormonal-lipid pathway of T2D showed evidence of positive causal relationships with CrC ($\beta_{MR} = 0.099$[SE = 0.269], P-value = $2.32x10^{-4}$) and BrC ($\beta_{MR} = 0.0937$[SE = 0.0354], P-value = 0.008), and a negative causal effect on PanC ($\beta_{MR} = -0.476$[SE = 0.182], P-value = 0.00872). Our analysis further suggested a causal

relationship between increased T2D risk via the glycaemic pathway and PrC ($\beta_{MR} = 0.131$[SE = 0.066], P-value = 0.0464).

Conclusions: Dissection of T2D risk variants into distinct pathways improved our ability to detect causal relationships between specific T2D pathways and cancers and highlighted hormonal-lipid- and glycaemia-related mechanisms underlying these relationships.Funding: WCRF-2017/1641, LongITools H2020-SC1-2019-874739

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P17.078.A Leveraging omic features with F3UTER enables identification of unannotated 3'UTRs for synaptic genes

Siddharth Sethi[1,2], *David Zhang*[2,3,4], *Sebastian Guelfi*[2,5], *Zhongbo Chen*[2,3,4], *Sonia Garcia-Ruiz*[2,3,4], *Mina Ryten*[2,3,4], *Harpreet Saini*[1], *Juan A. Botia*[2,6]

[1]*Astex Pharmaceuticals, Cambridge, United Kingdom,* [2]*Department of Neurodegenerative Disease, Institute of Neurology, University College London, London, United Kingdom,* [3]*NIHR Great Ormond Street Hospital Biomedical Research Centre, University College London, London, United Kingdom,* [4]*Genetics and Genomic Medicine, Great Ormond Street Institute of Child Health, University College London, London, United Kingdom,* [5]*Verge Genomics, South San Francisco, CA, USA,* [6]*Department of Information and Communications Engineering, University of Murcia, Murcia, Spain.*

The 3' untranslated regions (3'UTRs) of protein-coding messenger RNAs (mRNAs) play a crucial role in regulating gene expression at the post-transcriptional level. There is growing evidence for the importance of 3'UTR dependent regulatory processes, particularly in large polarised cells such as neurons. However, 3'-end RNA-sequencing datasets have identified a large number of novel polyadenylation sites, many of which are located outside of annotated exons, suggesting that our current 3'UTR catalogue in human is incomplete. These insights are complemented by an increasing recognition of the functional importance of transcriptional activity outside of known exons, particularly in human brain tissues. In this study, we developed a machine learning-based framework, leveraging both genomic and tissue-specific transcriptomic features to predict previously unannotated 3'UTRs. We identify unannotated 3'UTRs associated with 1,513 genes across 39 human tissues, with the greatest abundance found in brain. These unannotated 3'UTRs were significantly enriched for RNA binding proteins (RBPs) and exhibited higher human lineage-specificity than expected by chance. We found that brain-specific unannotated 3'UTRs were enriched for the binding of important neuronal RBPs such as *TARDBP* and *RBFOX1*, and their associated genes were involved in synaptic function and brain-related disorders. Our data is shared through an online resource F3UTER (https://astx.shinyapps.io/F3UTER/), which allows users to both easily query unannotated 3'UTRs and inspect the omic features driving the classifier's prediction. Overall, our data improves 3'UTR annotation and provides novel insights into the mRNA-RBP interactome in the human brain, with implications for our understanding of neurological and neurodevelopmental diseases.

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P17.079.B NGS-based detection of uniparental disomy in rare disease patients

Robert Schwieger, *Anett Marais, Joe Rayner, Antonio Romito, Paknia Omid, Volkmar Weckesser, Peter Bauer, Krishna Kumar Kandaswamy*

Centogene, Berlin, Germany.

Uniparental disomy (UPD) is a copy-neutral genetic defect where both chromosomal homologs originate from a single parent. UPDs are relatively rare (Nakka et al. 2019;Robinson 2000). However, increased prevalence was observed in patients with certain rare diseases (King et al. 2014). Here, we assess the pertinence of UPD-events in the context of rare disease diagnostics. Even though UPDs do not necessarily have a pathogenic effect an increased disease risk due to imprinting effects is reported. Iso-UPDs may encompass homozygous pathogenic variants. Furthermore, UPDs can indicate chromosomal aberrations due to incomplete rescue. We integrated automated UPD detection in our rare disease diagnostic workflow for Whole exome sequencing (WES) samples. We investigate the UPD distribution among rare disease patients. In particular, we are interested in predominant occurrence with respect to chromosomal location as well as expanding the direct diagnostic links for rare genetic disorders (Del Gaudio et al. 2020). Detection of UPDs is based on different methods such as MLPA-methylation, detection of Mendelian inheritance errors (MIEs) in family-trios (Yauy et al. 2020) or on the detection of ROHs (Magi et al. 2014). Here we combine different approaches and extend the detection method based on MIEs using approaches from Baysian machine learning. So far we tested around 3000 WES-trio samples and identified four previously unknown diagnostically relevant UPD events, three iso-UPDs on chromosomes 9, 15 and 16 and one hetero-UPD on chromosome 21. Furthermore, we developed a method for detection of UPDs in WES-solo cases and recently integrated it into the diagnostic pipeline.

R. Schwieger: A. Employment (full or part-time); Modest; Centogene. **A. Marais:** A. Employment (full or part-time); Modest; Centogene. **J. Rayner:** A. Employment (full or part-time); Modest; Centogene. **A. Romito:** A. Employment (full or part-time); Modest; Centogene. **P. Omid:** A. Employment (full or part-time); Modest; Centogene. **V. Weckesser:** A. Employment (full or part-time); Modest; Centogene. **P. Bauer:** A. Employment (full or part-time); Modest; Centogene. **K.K. Kandaswamy:** A. Employment (full or part-time); Modest; Centogene.

P17.080.C ConVarT: a new search engine for orthologous variants for functional inference of human genetic variants

Mustafa S. Pir[1], *Halil I. Bilgin*[1], *Ahmet Sayıcı*[1], *Fatih Coşkun*[1], *Furkan M. Torun*[1], *Pei Zhao*[2], *Yahong Kang*[2], *Sebiha Çevik*[1], *Oktay I. Kaplan*[1]

[1]*Abdullah Gul University, Kayseri, Turkey,* [2]*SunyBiotech Co., Ltd, Fuzhou, China.*

Introduction: Next-generation sequencing technologies have facilitated the sequencing of genomes of human and non-human organisms, leading to an immense amount of variant data. All of these variant data are stored in organism-specific databases, but finding equivalent variants called orthologous variants (OrthoVars) between organisms remains difficult.

Materials and Methods: We conducted over 500000 multiple sequence alignments, accompanied by the incorporation of variant-specific annotations. We determined OrthoVars between human, mouse or *C. elegans* and inserted them into corresponding positions. Result: Here, we developed a novel integrated search engine ConVarT (http://www.convart.org/), which includes human variants together with variants from mouse and *C. elegans*. Besides, ConVarT incorporated variant-specific annotations including pathogenicity and phenotypic consequences, thus helping to infer the functional

implications of human variants from phenotypic OrthoVars from non-human species. Furthermore, phenotypic variants without human OrthoVars from mouse and *C. elegans* may provide ready to use empirical evidence when human OrthoVars emerge.

Conclusion: ConVarT is a new search engine for OrthoVars between humans and non-human species, allowing the functional inference of human genetic variants from phenotypic OrthoVars and variants from non-human organisms.

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P17.081.D Systematic benchmarking of multiple variant calling pipelines identifies major factors affecting accuracy of coding sequence variant discovery

Yury A. Barbitoff[1,2], *Ruslan Abasov*[1], *Varvara E. Tvorogova*[1,3], *Andrey S. Glotov*[2], *Alexander V. Predeus*[1]

[1]Bioinformatics Institute, St. Petersburg, Russian Federation, [2]Dpt. of Genomic Medicine, D.O. Ott Research Institute of Obstetrics, Gynaecology, and Reproductology, St. Petersburg, Russian Federation, [3]Dpt. of Genetics and Biotechnology, St. Petersburg State University, St. Petersburg, Russian Federation.

Introduction: Accurate detection of genetic variants is a key requirement for molecular diagnostics of Mendelian disorders. Efficiency of variant discovery from NGS data depends on multiple factors, including the performance of read alignment and variant calling algorithms.

Methods: In this work, we systematically evaluated the performance of 4 short read aligners (bowtie2, BWA, Isaac, and Novoalign) and 6 variant calling and filtering methods (based on DeepVariant, GATK, FreeBayes, and Strelka) using a set of 10 "gold standard" WES and WGS datasets.

Results: Our results suggest that bowtie2 performs significantly worse than other aligners and should not be used for medical variant calling. When other aligners were used, the accuracy of variant discovery mostly depended on variant caller and not read aligner. Among the tested callers, DeepVariant consistently showed the best performance and the highest robustness on both WES and WGS data. All tested variant callers performed worse in regions with higher fraction of multimapping reads, and mappability and GC content were the greatest sequence-based confounders for all variant callers. At the same time, tools that showed best performance also displayed lower dependence on sequence-based confounders, sequencing technology (WES vs WGS), and coverage.

Conclusions: Our findings suggest that recent developments in variant caller software could compensate for most of the inherent limitations of short read sequencing methods.

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P17.082.A GenOtoScope: Automated annotation of variants associated with hereditary hearing loss

Christian Landgraf[1], *Damianos P. Melidis*[2], *Gunnar Schmidt*[1], *Anja Schöner-Heinisch*[1], *Sandra von Hardenberg*[1], *Bernd Auber*[1], *Wolfgang Nejdl*[2]

[1]Hannover Medical School (MHH), Hannover, Germany, [2]L3S Research Center, Leibniz University Hannover, Hannover, Germany.

The establishment of next generation sequencing (WES/WGS) in clinical routine has opened the diagnostic field for common disorders associated with a vast genetic heterogeneity like hearing loss (HL). The introduction of the "Expert Specification of ACMG/AMP Variant Interpretation Guidelines for Genetic Hearing Loss" (Oza et al., 2018) paved the way for a more standardized assessment. However, congruent interpretation of a large number of genomic variants remains a key challenge in today's molecular genetics, which also stands true for HL. Thus, automated variant (pre-)assessment could be an important structural benefit, as analyses are still time-consuming and prone to inconsistent interpretation. Here we present *GenOtoScope*, a software pipeline that accepts the patient genomic variants (vcf file) as input and computes the ACMG class for each variant, using Python programming language. Currently, we have implemented 15 out of 24 ACMG/AMP rules specified for HL on different strength levels including the refined recommendations for PVS1 interpretation (Tayoun et al., 2018). We have successfully tested *GenOtoScope* for exonic variants of 16 individuals with HL. Further, we aim to test and evaluate the pipeline for a broader set of patients. Finally, the project aims to provide a well-documented and easy-to-run pipeline, which automatically assigns a class to each variant and presents only a handful set of the relevant variants to the human curator. Thus, we aim that *GenOtoScope* will standardize the process and significantly decrease the time of variant assessment and interpretation for HL. Funded by Volkswagen Foundation.

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P17.083.B Variant Interpretation Pipeline: a modular pipeline that integrates best practice methods to prioritize genetic variants causal for a patient's phenotype

Lennart F. Johansson, *K. Joeri van der Velde, Dennis Hendriksen, Bart Charbon, Mariska K. Slofstra, Robert Sietsma, Sander Van den Hoek, Martine T. Meems-Veldhuis, Henny H. Lemmink, Mariëlle E. van Gijn, Cleo C. Van Diemen, Morris A. Swertz*

UMCG, Groningen, Netherlands.

Thus far, almost 7000 diseases with a molecular basis are defined, of which almost 6000 single gene disorders, and around 4000 genes are known to harbor the pathogenic variants causal for the patient's phenotypes. Still, the diagnostic yield of genetic testing varies between 24-68%, depending on patient inclusion criteria, whether trio's are studied, patient's phenotype(s) and analysis strategies. Fortunately new methods are published frequently, however their timely implementation necessitates a flexible analysis workflow. Therefore we present MOLGENIS Variant Interpretation Pipeline (VIP), a flexible system to prioritize genetic variants in a VCF on the likelihood to be causal for a patient's phenotype. VIPs main added value is that it is modular and configurable, integrating best in class software, such ENSEMBL Variant Effect Predictor, SpliceAI, CADD, gnoMAD, CAPICE and VIBE, with validated decision protocols from routine diagnostics practice and integrating inputs from experts from EU Solve-RD, EJP-RD and CINECA projects. VIP provides annotation, prioritization and filtering of variants through GENMOD-based inheritance matching and HPO-based phenotype matching, including SV, low-penetrance and GRCh38 pilots. Using these options and annotations users can create custom filter trees to classify variants. Moreover, interactive HTML reports can be generated to filter and

select variants of interest. We believe VIP helps get the best analysis methods to patients, faster. VIP is open source, and we welcome community contributions to add novel tools and create new pipeline configurations suited to different use-cases. Find the latest release here: https://github.com/molgenis/vip/releases and https://github.com/molgenis/vip-report/releases.

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P17.085.D MobiDetails: online DNA variants interpretation

David BAUX[1,2], *Charles Van Goethem*[1], *Olivier Ardouin*[3], *Thomas Guignard*[4], *Anne Bergougnoux*[1,5], *Michel Koenig*[1,5], *Anne-Françoise Roux*[1,2]

[1]*Laboratoire de génétique moléculaire, CHU Montpellier, Université de Montpellier, Montpellier, France,* [2]*Institute for Neurosciences of Montpellier (INM), Univ Montpellier, INSERM, Montpellier, France,* [3]*Plateau de Médecine Moléculaire et Génomique, CHU Montpellier, Université de Montpellier, Montpellier, France,* [4]*Unité de Génétique Chromosomique, CHU Montpellier, Université de Montpellier, Montpellier, France,* [5]*PhyMedExp, Univ Montpellier, INSERM, Montpellier, France.*

MobiDetails is an online expert tool dedicated to clinicians and researchers assessing DNA variants pathogenicity. This complex task is rendered even more difficult by the requirement to use several websites and tools to obtain exhaustive and appropriate data (i.e population genomics, missense and splicing predictors, literature citations…). In addition, some tools do not have a dedicated web interface or can be time consuming for the end-user. The aim of MobiDetails is to gather in a single web page for each variant the most significant data, with a particular focus on splicing prediction tools. It is based on publicly available resources and/or on open-source academic software (e.g. VariantValidator to generate the HGVS nomenclatures). It is able to annotate any small DNA variant (substitutions or small insertions/deletions), either exonic or intronic, lying within 18,500 human genes. MobiDetails annotations are dynamic, as they either rely on local files updated on a regular basis (e.g. ClinVar data) or on live API calls (e.g. to LOVD, LitVar for literature queries, Intervar or MetaDome). Therefore, updates of these tools are reflected in MobiDetails results and regular visits to key variant pages can bring new insights and help refine classification. MobiDetails is totally free for use to academics and does not require any account to annotate a new variant and browse the results. However registered users can record ACMG classifications for the variants, and trigger the automated submission of the variant to the Global Variome Shared LOVD instance, ensuring a persistent sharing of the variants. https://mobidetails.iurc.montp.inserm.fr/MD

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P17.086.A Phenotype based prediction of WES outcome using machine learning

Alexander J. M. Dingemans[1], *Max Hinne*[2], *Sandra Jansen*[1], *Jeroen van Reeuwijk*[1], *Nicole de Leeuw*[1], *Rolph Pfundt*[1], *Bregje W. van Bon*[1], *Anneke T. Vulto-van Silfhout*[1], *Tjitske Kleefstra*[1], *David A. Koolen*[1], *Marcel A. J. van Gerven*[2], *Lisenka E. L. M. Vissers*[1], *Bert B. A. de Vries*[1]

[1]*Radboudumc, Nijmegen, Netherlands,* [2]*Radboud University, Nijmegen, Netherlands.*

Although the introduction of whole exome sequencing (WES) has led to the diagnosis of a significant portion of patients with intellectual disability (ID), the yield in actual clinical practice has remained stable in the last few years at approximately 30%. We hypothesize that improving the selection of patients to test based on their phenotypic presentation will increase diagnostic yield and therefore reduce unnecessary genetic testing. In the current study, we tested four machine learning methods to predict the probability of a positive WES. From these, we developed PredWES: a statistical model predicting the probability of a positive WES result solely based on the phenotype of a patient. We first trained the tool on 1,431 patients with ID using nested cross-validation. We subsequently show that diagnostic WES on the top 10% of patients with the highest probability of a positive WES result would provide a diagnostic yield of 57%, leading to a notably 86% increase. On the other end of the spectrum, the 90% of patients with the 10% lowest scores were correctly predicted to have a negative WES result. Inspection of our model revealed that for patients with ID, comorbid abnormal (lower) muscle tone positively correlated with the prediction for a conclusive WES diagnosis, whereas autism was negatively associated with a molecular diagnosis. In conclusion, PredWES allows prioritizing patients eligible for diagnostic WES testing based on their phenotypic presentation to increase the relative diagnostic yield for intellectual disability, as such, making a more efficient use of health care resources.

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P17.087.B High-definition likelihood inference of heritability and genetic correlation on the X chromosome

Jiantao Chen[1], *Zheng Ning*[2], *Xia Shen*[1]

[1]*Sun Yat-sen University, Guangzhou, China,* [2]*Karolinska Institutet, Stockholm, Sweden.*

Estimating heritability and genetic correlation is of essential importance for understanding the genetic architecture of complex traits. Without accessing individual-level data, the state-of-the-art methods can infer these parameters using summary statistics from genome-wide association studies (GWAS), including linkage disequilibrium score regression (LDSC) and recently developed more powerful high-definition likelihood (HDL) methods. Most applications of these methods were limited to analyzing the autosomal variants, while the X chromosome was often neglected. Here we develop an extended HDL method, HDL-X, to analyze heritability and genetic correlation on the X chromosome. We applied HDL-X on 30 complex traits measured in about 155,000 unrelated British men and 180,000 unrelated British women from the UK Biobank (UKBB). In contrast to women, we found that men have enriched heritabilities across most phenotypes on the X chromosome. While for both sexes, the genetic correlations on the X chromosome are comparable to the autosomal estimates.

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P18 Personalized Medicine and Pharmacogenomics

P18.002.D THL Biobank, an infrastructure for multi-omics systems biology and personalized medicine studies

Heidi Marjonen, *Anni Joensuu, Katri Kantojärvi, Kaisa Silander, Markus Perola, Sirpa Soini*

Finnish Institute for Health and Welfare, Helsinki, Finland.

Introduction: Genomics-based knowledge is revolutionizing the practice of medicine by leading it to more effective diagnosis and treatment. Here biobanks can provide a valuable resource of samples to be linked with detailed descriptions of disease phenotypes and genetic data to support the research era of personalized medicine. THL Biobank is a remarkable biorepository of both population and disease-specific research collections to be used in research aiming to develop new solutions for health promotion and disease prevention.

Material and methods: Precision medicine research requires access to sufficient numbers of samples and data. THL Biobank provides access to >200 000 samples from population-based cohorts with standardized life style, anthropometrics and biomarker data as well as availability of clinical follow-up data form health registers. By combing this to genomic data (chip/imputed data >100 000 individuals, WES >15 000 individuals and WGS >5000 individuals) and other omics data of metabolome, methylome, transcriptome and telomeres we provide a fundamental scientific infrastructure for personalized medicine.

Results: THL Biobank serves both academic and company researchers worldwide and also offers a possibility for recall studies. Over 130 biobank projects have been initiated since 2015 and >94% included genomic data such as the large public-private genetic research study FinnGen.

Conclusions: Identification of disease specific biomarkers for diagnosis and prognosis is the key feature of precision medicine. THL Biobank is a resource for extensive data collections that offer tremendous research opportunities from gene-environment interactions and polygenic risk scores to new diagnostics.

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P18.003.A Association of bradikinin receptor genes (ins / del (9b)), chimase 1 (1903A> G), and FTO (rs9939609) with obesity in children and adolescence

Olga V. Bocharova[1], *Elena D. Teplyakova*[1], *Tatyana P. Shkurat*[2], *Maria A. Amelina*[2]

[1]*Rostov State Medical University, Rostov-on-Don, Russian Federation,* [2]*Southern Federal University, Rostov-on-Don, Russian Federation.*

Genetic predisposition plays an important role in the development of obesity, but the relationship between obesity loci and gene polymorphisms associated with cardiometabolic disorders in the child and adolescent population has not been established. Aim of this work **was** to study the relationship between polymorphisms of the genes *BDKRB2 ins/del (9b)*, *CMA1/B 1903A>G*, and the gene associated with obesity *FTO rs9939609* in the child and adolescent population Rostov region (Russia).

Methods: The survey involved 370 obese children and adolescents from 3 to 17 years old with a body mass index (BMI > 30), as well as 123 children and adolescents of the same age without obesity (BMI < 20). Genomic DNA samples were isolated from whole blood of patients using the standard phenol-chloroform method. Single nucleotide polymorphism was determined using polymerase chain reaction. MDR was used to assess gene-gene relationships. Distribution of genotypes and allelic variants/genotypes was analyzed using the $\chi 2$ test.

Results: An increased risk of obesity is shown for carriers of polymorphisms *rs1800875* of the *CMA1* gene and *rs9939609* of the *FTO* gene. Analysis of the two- and three-locus relationships showed an increased risk of obesity when all studied gene polymorphisms were included in its development. An antagonistic character was also established between all studied genes and combinations of combined genotypes of obesity risk in the child and adolescent population were identified. *This study was funded by the Ministry of Science and Higher Education of the Russian Federation №0852-2020-0028*.

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P18.004.B Personalising breast cancer prevention

Sowmiya Moorthie[1,2], *Hilary Burton*[1], ***Chantal Babb de Villiers***[1], *Tanya Brigden*[1], *Alison Hall*[1], *Laura Blackburn*[1], *Mark Kroese*[1]

[1]*PHG Foundation, Cambridge, United Kingdom,* [2]*Cambridge Public Health, University of Cambridge, Cambrige, United Kingdom.*

Introduction: Developing precision public health and personalised prevention is an ambition for many health systems as evidenced by government policy documents. This has become increasingly possible through the convergence of information technology and advances in our knowledge about risk, enabling more accurate risk estimation. Breast cancer is no exception and major scientific advancements have been made in risk prediction and the tools used. However, the pace at which this research is occurring is significantly quicker than implementation.

Aim and method: Our aim was to understand the opportunities for personalisation and how new knowledge from breast cancer prevention research could best be integrated into personalised prevention pathways.We reviewed the scientific and policy literature to gain an understanding of the present. We then convened an expert workshop to develop a vision of the future, enabling us to examine opportunities for implementation and identification of issues relevant to individuals, health systems and society. We used our knowledge gathering and policy analysis to develop recommendations that inform policymakers on personalised breast cancer prevention pathways.

Conclusion: Our report, *Personalising breast cancer prevention - bridging the gap between research and policy* summarises the overarching implications for breast cancer prevention pathways for policymakers, those working in health promotion, and healthcare providers. We provide recommendations for important areas of decision-making and considerations for advancing personalised breast cancer prevention pathways. Recommendations included relate to: Accelerating the use of new tools in preventing hereditary breast cancer, the use of these tools in breast cancer prevention pathways, and moving towards risk-stratified population screening.

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P18.005.C Detection of pathogenic mutations in breast cancer genes by GSAMD and PMDA array platforms

Annemieke J. M. H. Verkerk, *Jeroen van Rooij, Jard de Vries, Ans M. W. van de Ouweland, Maartje J. Hooning, Linda Broer, Willemina R. R. Geurts-Giele, Robert M. van der Helm, Robert M. W. Hofstra,*

Marieke F. van Dooren, J Margriet Collée, E H. Hoefsloot, Andre G. Uitterlinden, Joyce B. van Meurs

Erasmus MC, Rotterdam, Netherlands.

Introduction: At our EMC we have initiated the GOALL project (Genotyping On All Patients), in which we investigate the use of high throughput SNP arrays for improving clinical care and making personalized medicine available to a larger public. In this pilot we compared the performance of array-based genotyping with previously clinically determined pathogenic mutations, for 240 breast cancer cases. Diagnostic results were based on Sanger sequencing (SNVs and small indels ≤ 50 bp) and MLPA (deletions or duplications > 50 bp) for the associated genes BRCA1, BRCA2, CHEK2, PALB2 and ATM.

Materials and Methods: Cases were selected who carried mutations that were present on the arrays and then genotyped on the Illumina GSAMD and Thermofischer PMDA arrays, without prior knowledge for the research group which mutations would be included. After data processing and QC, samples were called for SNVs using the manufacturer's software and analyzed by PENNCNV and NEXUS (Biodiscovery) to assess larger deletions or duplications.

Results: Overall 90% of the present diagnostic mutation results were confirmed by array. No false positives were detected. Mutations that were missed were mainly due to technical issues on probe design or resolution and will we further discussed.

Conclusion: We evaluate the suitability of SNP arrays for detecting rare pathogenic mutations in breast cancer genes. Although new small mutations will be missed, this is a low cost and effective way to screen for mutations in larger populations. Additionally these arrays can be customized to include extra variants for a higher detection rate.

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P18.006.D Carrier frequency of four common recessive diseases in a Russian population

*Anna Kiseleva[1], Evgeniia Sotnikova[1], Alexandra Ershova[1], Vladimir Kutsenko[1], Marina Klimushina[1], Mikhail Divashuk[1], Olga Skirko[1], Olga Kurilova[1], Anastasia Zharikova[1,2], Vasily Ramensky[1,2], Irina Efimova[1], Petr Slominsky[3], Svetlana Shalnova[1], **Alexey Meshkov**[1], Oxana Drapkina[1]*

[1]National Medical Research Center for Therapy and Preventive Medicine, Ministry of Healthcare of the Russian Federation, Moscow, Russian Federation, [2]Faculty of Bioengineering and Bioinformatics, Lomonosov Moscow State University, Moscow, Russian Federation, [3]Institute of Molecular Genetics, Russian Academy of Sciences, Moscow, Russian Federation.

Introduction: Genetic carrier screening is an advanced tool for reducing recessive disease burden. In order to ensure its effective work it is important to improve the knowledge of population genetic structure. Therefore, the aim of this study was to conduct comparison of carrier frequency for cystic fibrosis (CF), phenylketonuria, alpha-1 antitrypsin deficiency, and sensorineural hearing loss (SNHS) in two geographically close Russian regions.

Materials and Methods: The custom panel consisting of 116 variants in *CFTR, PAH, SERPINA1*, and *GJB2* genes was designed and tested on two population-based cohorts that included 1858 ESSE-Ivanovo and 1244 ESSE-Vologda participants. Genotypes were determined by NGS sequencing on Nextseq 550 and by Real-Time PCR with TaqMan OpenArray assays, respectively.

Results: Detected carrier frequencies (DCFs) differed significantly between two regions only for CF: 0.0156 in ESSE-Ivanovo and 0.0299 in ESSE-Vologda ($p = 0.009$). For phenylketonuria DCFs were 0.0226 and 0.0239; for alpha-1 antitrypsin deficiency - 0.0436 and 0.0497; for SNHS - 0.0576 and 0.0696, respectively. Contribution to the DCFs differences for CF was made by the most common *CFTR* variant (p.F508del) with allele frequency 0.5% in ESSE-Ivanovo and 0.9% in ESSE-Vologda, and by rare variants.

Conclusions: Carrier frequencies for these common recessive diseases were obtained for the first time in two cohorts of the Russian population. The obtained frequencies confirm the feasibility of genetic carrier screening for all four studied diseases.

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P18.008.B *ELOVL7* gene region as s potential risk locus for adalimumab response in Slovenian patients with Crohn's disease

***Mario Gorenjak**[1], Mateja Zupin[1], Gregor Jezernik[1], Pavel Skok[2,1], Uroš Potočnik[1]*

[1]Faculty of Medicine, Maribor, Slovenia, [2]University Clinical Centre Maribor, Maribor, Slovenia.

Introduction: Response to anti-TNF therapy is of pivotal importance in patients with Crohn's disease. We integrated previously reported and our PBMC derived transcriptomic and genomic data for identification of genetic biomarkers for discrimination between responders and non-responders to anti-TNF therapy.

Materials and Methods: 84 Crohn's disease patients naïve to adalimumab treatment were enrolled in the present study. DNA and RNA were extracted from peripheral blood mononuclear cells. RNA-seq with deconvolution was performed using BGISEQ-500. Genotyping was performed using Infinium Global Screening Array. Association regressions were carried out with 12-week response to adalimumab as an outcome variable, adjusted to age at diagnosis, sex, concomitant treatment and principal components. Mendelian randomization was performed using association and eQTL results. Results were subsequently validated using RT-qPCR.

Results: *ELOVL7* gene region was confirmed using integration of RNA-seq ($q = 0.035$; Log_2FC: 1.55) and analysis of single nucleotide variants in ± 100kb *ELOVL7* region. Functional analysis has shown that most significant rs78620886 ($p = 3 \times 10^{-4}$) is listed at H3K9ac_Pro histone modification epigenetic mark in HaploReg database. Moreover, mendelian randomization with eQTL integration has confirmed the involvement of *ELOVL7* ($p = 0.046$) with adalimumab response. RT-qPCR validation additionally confirmed statistically significant higher expression of *ELOVL7* in non-responders ($p = 0.016$; FC: 1.43).

Conclusions: The present study confirmed *ELOVL7* involvement in anti-TNF response and revealed that the regulation of genes in adalimumab response may be a part of a complex interplay of -omics. Funding: This work was financially supported by the project ID J3-9258 from the Slovenian Research Agency and Ministry of Education, Science and Sport C3330-19-952026.

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P18.010.D *CTNNA1* as a Hereditary Diffuse Gastric Cancer predisposing gene

Silvana Lobo[1,2,3], *Carla Oliveira*[1,2,4]

[1]*Instituto de Investigação e Inovação em Saúde (I3s), Porto, Portugal,* [2]*Instituto de Patologia e Imunologia da Universidade do Porto (IPATIMUP), Porto, Portugal,* [3]*Programa Doutoral em Biotecnologia Molecular e Celular Aplicada às Ciências da Saúde do Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, Porto, Portugal,* [4]*Faculdade de Medicina da Universidade do Porto (FMUP), Porto, Portugal.*

Introduction: Hereditary Diffuse Gastric Cancer (HDGC) predisposes for diffuse gastric cancer (DGC) and/or lobular breast cancer (LBC). HDGC is mainly caused by *CDH1*/E-cadherin loss of function. Recently *CTNNA1*, encoding α-E-catenin, an E-cadherin adherens-junctions partner became a HDGC-associated gene.

M&M: We systematically reviewed the literature searching for *CTNNA1*-germline variant carriers. We classified carriers based on HDGC clinical criteria and variants according to *CDH1*/ACMG/AMP guidelines and performed genotype-phenotype analysis.

Results: 41 families with 105 family-members were found to carry *CTNNA1*-germline variants. All probands from 13 HDGC-families presented DGC (average≈40yo), as most relatives. 10/13 (76.9%) carried pathogenic (P) variants. Most probands from 28 non-HDGC-families developed unspecified BC (average≈51yo) as their relatives (average≈56yo). 4/28 (14.3%) carried P, while 11/28 (39.3%) carried likely P (LP) variants. When considering phenotypes in P and LP variant carrier families, independently of clinical criteria, we found that 100% of DGC and unspecified-GC (32/32) clustered in the P-variant group, while 84% (21/25) of unspecified-BC clustered in the LP variant group. Only 2/105 of the full cohort presented LBC. Non-HDGC-families presented a truncating variant cluster in *CTNNA1* last exon.

Discussion: Herein, we confirm the association between *CTNNA1* P variants and early-onset GC, mainly DGC, but not LBC; the lack of HDGC-related phenotypes in LP variant carriers; and suggest a limited deleteriousness for truncating variants in *CTNNA1* last exon. Our results support using *CDH1*/ACMG/AMP guidelines for *CTNNA1* variant classification and considering restricting clinical actionability to *CTNNA1* variants classified as P.

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P18.011.A Preemptive targeted pharmacogenomic testing with Axiom PharmacoFocus Array

Carsten Bruckner, *Manasi Shah, Debbie Black, Mirjana Marjanovic*

Thermo Fisher Scientific, Santa Clara, CA, USA.

Introduction: Understanding common variations in genes coding for drug metabolism and transport proteins can provide insight in medication management research to reduce adverse drug reactions and improve health outcomes. Early integration of pharmacogenomic (PGx) information in clinical research enables better understanding of participant drug response, leading to optimal research outcomes. The Applied Biosystems™ Axiom™ PharmacoFocus™ Array provides a targeted, high throughput and cost-efficient solution for preemptive PGx research in labs, academic hospitals and health care centers. It offers comprehensive coverage of high-evidence functional variants (Pharmacogenomics Knowledge Base annotation levels of evidence 1A-2B) that influence absorption, distribution, metabolism, and excretion (ADME) of common medications.

Materials and Methods: A verification study tested approximately 1000 DNA samples across three sites from multiple DNA sources including blood, saliva, and buccal swabs. A novel multiplex PCR step was incorporated into the Axiom™ workflow to address the genotyping challenges of key variants that are part of highly homologous multi-gene families such as CYP2D6. A new analysis algorithm was used to more accurately measure copy number changes in pharmacogenomically relevant genes. Axiom™ Analysis Suite was used to convert genotype calls to recognized star nomenclature and predicted phenotype.

Results: Analytical performance was evaluated on overall call rate (>99%) and concordance to independent genotypes (>99.8% vs 1000 Genomes Project Phase III). Copy number changes in pharmacogenomically relevant genes were compared to consensus calls for those regions, with concordance >99.7%.

Conclusions: The Axiom PharmacoFocus Array can accurately genotype even technically challenging ADME variants in complex genes.

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P18.012.B Full-length sequencing of *CYP2D6* locus with HiFi reads increases genotyping accuracy

Lei Zhu, *Aaron Wenger, Josiah Wilcots, Primo Baybayan*

Pacific Biosciences, Menlo Park, CA, USA.

Introduction: The highly polymorphic *CYP2D6* gene impacts the metabolism of 25% of the mostly prescribed drugs. Thus, accurate identification of variant *CYP2D6* alleles in individuals is necessary for personalized medicine. PacBio HiFi sequencing produces long and accurate reads to identify variant regions. Here, we describe an end-to-end workflow for the characterization of full-length *CYP2D6* by HiFi sequencing.

Materials and Methods: The 2-step long-range PCR was developed for the amplification of *CYP2D6* locus and applied to 22 samples from a pharmacogenomics reference panel. Barcoded amplicons were pooled together for the preparation of a SMRTbell library, which was sequenced on the PacBio Sequel II and IIe Systems. HiFi reads were demultiplexed and a consensus of each haplotype was generated, mapped to the human reference genome, and assigned a diplotype.

Results: More than 700,000 full-length HiFi reads with an average read length of 8.2 kb at a mean accuracy ≥99.9% were generated per sequencing run. Nearly all (>99%) demultiplexed reads were on target to *CYP2D6*. For 21 of 22 samples, the diplotypes revealed from HiFi reads matched the reference genotypes, while providing full resolution of each allele. For one sample characterized previously as *1/*41 by microarray, PacBio HiFi data produced a corrected type of *33/*41. In addition, for 4 of 22 samples HiFi sequencing identified duplications missed by microarray or real-time PCR.

Conclusions: Our results demonstrate that SMRT Sequencing generates full-length HiFi reads, providing high resolution for accurate detection of the polymorphic *CYP2D6* locus.

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P18.013.C Phasing of the entire *CYP2D6* locus with CRISPR-Cas9 enriched Nanopore sequencing

Laurentijn Tilleman, *Kaat Rubben, Filip Van Nieuwerburgh*

Ghent University, Laboratory of Pharmaceutical Biotechnology, Ghent, Belgium.

Introduction: *CYP2D6* is a highly polymorphic gene, with more than hundred star(*)-allele haplotypes and more than a dozen structural variants, including copy number variation, gene deletions, and gene hybrids. Today, many genotyping and sequencing platforms are developed to determine these variants. However, none of them can detect all possible star-allele haplotypes and structural variants without introducing PCR or hybridization mediated errors. In this study, a PCR-free enrichment method is used to sequence the entire *CYP2D6* locus, including surrounding genes.

Material and methods: To target the *CYP2D6* locus, nine guide RNAs were designed: four were located upstream of *CYP2D6*, three in the region between *CYP2D6* and *CYP2D7*, and two downstream of *CYP2D7*. The performance of these guide RNAs was tested on DNA from the NA12878 cell line. CRISPR-Cas9 enrichment was performed according to the Cas-mediated PCR-free enrichment Protocol from Oxford Nanopore Technologies and sequenced on an R9.4.1 flowcell. Sequencing data was basecalled with Guppy 4 and mapped with minimap2. Downstream analysis was done using custom python scripts.

Results: The *CYP2D6* locus was enriched 130 times, with a mean coverage of 290X. Eight reads span the entire *CYP2D6* locus. All known variants were called correctly and could be phased correctly as well. One additional INDEL and three additional SNPs were detected.

Conclusion: This technique offers a high-throughput method for accurate haplotype and structural variant detection of known and unknown variants of *CYP2D6* and *CYP2D6-CYP2D7* hybrids.

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P18.014.D Very rare or yet unknown *CFTR* variants in pediatric patients suspicious for Cystic Fibrosis

Julia Hentschel[1], *Constance Henn*[2], *Maike Karnstedt*[1], *Simon Gräber*[3,4], *Freerk Prenzel*[2]

[1]*Institute of Human Genetics, Leipzig, Germany,* [2]*Hospital for Children and Adolescents, University of Leipzig Medical Center, Leipzig, Germany,* [3]*Department of Pediatric Pulmonology, Immunology and Critical Care Medicine and Cystic Fibrosis Center, Charité - Universitätsmedizin Berlin, Berlin, Germany,* [4]*Berlin Institute of Health (BIH), Berlin, Germany.*

Despite enhanced knowledge about CFTR genetic confirmation of diagnosing Cystic Fibrosis (CF) sometimes remain difficult due to variants of unknown significance (VUS). We present four patients clinically or in newborn screening (NBS) conspicuous for CF with very rare or unknown *CFTR* variants. Index 1 is a male newborn with meconium plug syndrome showed normal values in NBS. After failure to thrive and severe hypochloremic alkalosis sweat chloride (SC) was found pathologic. *CFTR* sequencing revealed compound-heterozygous pathogenic variants F508del and p.(Ala1087Pro). The 2nd variant was described only once, a dominant negative effect was assumed but not proven. In index 2, a female newborn with positive NBS and pathologic/intermediate SC, genetics revealed compound-heterozygosity for p.(Arg1162*) and a duplication of uncertain significance of exon 22 of *CFTR*. Besides a decreased pancreatic function no further CF-typical symptoms occurred to date. Index 3 was clinically conspicuous for CF at the age of 2 years with failure to thrive and severe exocrine pancreatic insufficiency. SC was intermediate/normal but we detected the variable variant 5T-12TG and a compound-heterozygous unknown VUS p.(Ile231Thr). Index 4 was diagnosed with CF after pulmonary infections, pathologic SC and compound heterozygosity for F508del and p.(Ala1319Glu). The 2nd variant is highly likely to cause CF, but still remain as VUS due to lacking functional data. In VUS functional analyses of CFTR channel is of importance not only to prove the diagnosis but also to give perspective to parents and clinicians, especially regarding to the possibility of CFTR modulator therapy.

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P18.015.A Integrated left ventricular global microRNA and mRNA profiling in human idiopathic dilated cardiomyopathy

Dilek Colak[1], *Olfat Al-Harazi*[1], *Ibrahim H. Kaya*[2], *Nzioka P. Muiya*[1], *Namik Kaya*[1], *Nduna Dzimiri*[1]

[1]*King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia, Riyadh, Saudi Arabia,* [2]*AlFaisal University, Riyadh, Saudi Arabia.*

Introduction: The triggering factors for the disease pathways leading to idiopathic dilated cardiomyopathy (DCM) are still elusive. MicroRNAs are short non-coding endogenous messenger RNAs that regulate gene expression post-transcriptionally. Hence, alterations in their expression may influence DCM disease pathways. In the present study, we sought to identify significantly altered miRNAs and genes involved in DCM by integrating left ventricular myocardial genome-wide microRNA and mRNA expression profiling and explore the mechanisms underlying the disease.

Materials and Methods: We performed expression profiles of DCM ($n = 15$) and control ($n = 12$) samples from left ventricle (LV) using array-based techniques and integrated the differentially expressed miRNAs with the global mRNA expression profiling. The gene signature was then validated using independent datasets of gene expression profiling data. Moreover, functional, gene ontology and network analyses were performed.

Results: We identified 33 significantly altered miRNAs in DCM and performed unsupervised principal component analysis and hierarchical clustering using the target genes that resulted from the integration of differentially expressed miRNAs and mRNAs. We then explored relevant transcriptomic and molecular networks and validated the diagnostic value of the microRNA signature using independent datasets.

Conclusions: Our study revealed several miRNAs that may be involved in various gene regulatory functions in DCM, which may provide robust biomarker panels for the disease.

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P18.016.B Direct-to-consumer genetic tests providing health information: A systematic review of consequences for consumers and healthcare services

Joshua J. Nolan[1,2], *Elizabeth Ormondroyd*[3,4]

[1]*Oxford University Hospitals NHS Foundation Trust, Oxford, United Kingdom,* [2]*University of Manchester, Manchester, United Kingdom,* [3]*University of Oxford, Oxford, United Kingdom,* [4]*Oxford Biomedical Research Centre, Oxford, United Kingdom.*

Introduction: Direct-to-consumer genetic tests (DTC-GT) can provide health-related information outside clinical care pathways and are widely available. Using systematic review methodology, we have sought to understand the consequences of commercially available genetic tests on consumers, patients, and healthcare services.

Methods: We conducted a systematic review of the literature, including qualitative, quantitative, and mixed-methods, in addition to case reports published since November 2015. PRISMA guidelines and a prospectively registered review protocol were used. A thematic synthesis was undertaken to identify major analytical themes.

Results: Forty-three papers met full inclusion criteria. Most consumers are satisfied with DTC-GT and trust their results, but do not complete as many post-test actions (sharing results, accessing healthcare, and changing behaviours) as they intended to. A small proportion of consumers are left dissatisfied, have negative experiences or are adversely impacted. Consumers are increasingly accessing third-party interpretation software to have their raw data re-analysed. False positive rare genetic variants are a significant problem. Healthcare professionals (HPs) feel a duty towards DTC-GT consumers as patients, yet some feel managing patients with DTC-GT is not an appropriate use of their time, impacts resource allocation and adds to HP workload. Some HPs feel DTC-GT could be of benefit for ancestry, but less so for health-related information. Some HPs perceive consumer/patients' understanding of genetics and trust in genetic professionals could be compromised by DTC-GT.

Conclusions: DTC-GT health-related information presents diverse challenges to consumers and healthcare services and may contribute to healthcare inequities. Third-party interpretation platforms further challenge stakeholders.

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P18.017.C A molecular approach to precision medicine in South African children with epilepsy: towards a genetics-based diagnostic service for epilepsy in childhood

Caitlin Mary McIntosh[1], *Karen Fieggen*[1], *Jo M. Wilmshurst*[2,3], *Alina I. Esterhuizen*[1,4]

[1]*Division of Human Genetics, Department of Pathology, Institute of Infectious Diseases and Molecular Medicine, University of Cape Town, Cape Town, South Africa,* [2]*School of Child and Adolescent Health, University of Cape Town, Cape Town, South Africa,* [3]*Paediatric Neurology and Neurophysiology, Red Cross War Memorial Children's Hospital, Cape Town, South Africa,* [4]*National Health Laboratory Service, Groote Schuur Hospital, Cape Town, South Africa.*

Introduction: Epilepsy is a neurological disorder characterised by unprovoked, recurring seizures, which affects more than 70 million people worldwide, the vast majority of which are residents of lower- and middle-income countries. Many cases of epilepsy previously deemed idiopathic have now been found to have a genetic cause, and genetic diagnosis of epilepsy can have a major impact on treatment and improve prognoses. A means of genetic diagnosis for epilepsy is unavailable to many people in the South African public health system.

Materials and Methods: A next-generation sequencing-based gene panel was designed making use of relevant literature and information from commercially available gene panels. A total of 78 genes were selected for inclusion in the gene panel. A cohort of 40 children with complex epilepsy had been previously recruited, and they underwent sequencing using Ion Torrent sequencing technology. Results from NGS were confirmed using traditional Sanger sequencing methods.

Results: Next-generation sequencing of the gene panel was successful and identified 24 variants in 20 probands for further analysis. Preliminary Sanger results have confirmed that the NGS panel is identifying true variants, but substantial drop off of putative variants was observed after segregation analysis. Variants were primarily identified in autosomal dominant and X-linked genes.

Conclusion: Preliminary results suggest this panel is successfully identifying potentially pathogenic variants in children with complex epilepsy. The low prevalence of variants in autosomal recessive genes suggests further research in African populations is necessary to identify non-*de novo* epilepsy-causing variants in those populations.

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P18.020.B Genetically based personalized approach to patients with metabolic and eating disorders - a case study

Victoria L. Spasova[1,2], *Boryana Gerasimova*[2], ***Olga S. Antonova***[1,2]

[1]*Department of Medical Genetics, Medical University of Sofia, Sofia, Bulgaria,* [2]*Re:Gena, Sofia, Bulgaria.*

Background: For the last 20 years a large amount of data has been gathered showing the genetic predisposition to overweight and obesity. The aim of this study is to demonstrate the personalized, genetic-based approach to normalize patients' weight and eating habits.

Materials & Methods: Eight patients were enrolled in the study aged 28-51. A set of 11 single nucleotide polymorphism (SNPs) related to lipid metabolism, absorption, insulin sensitivity, regulation of postprandial glucose level, sweet tooth, eating disorders and addictions: *APOA2* (rs5082), *ADIPOQ*, (rs17300539), *FTO* (rs9939609), *KCTD10* (rs10850219), *LIPC* (rs1800588), *MMAB* (rs2241201), *PPARG* (rs1801282), *ANKK1/DRD2* (rs1800497), *TAS2R38* (rs1726866), *LEPR* (rs2025804) and *SLC2A2* (rs5400). Based on the genetic results, the type of diet: balanced, Mediterranean, low-fat and low-carbohydrate is determined. The predisposition to unhealthy eating habits is described. The genetic counselling is performed prior to dietitian advice to build a personalized diet.

Results: The BMI of the studied patients ranges from 17.58 to 38.95 kg/m^2. According to the BMI they are divided into four groups – underweight (n = 2), normal weight (n = 2), overweight (n = 2), and obesity (n = 2). The results obtained, show that the patients' diet is so far equivocally different from the genetically determined one. Il patients, except one, have predispositions to particular unhealthy eating habits.

Conclusion: This small cohort demonstrates well established personalized approach that could be used not only for the daily nutritional habits optimization but also for prevention of the polygenic-multifactorial socially significant diseases.

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P18.021.C Using correlation information in precision medicine

Palle Duun Rohde[1], *Peter Sorensen*[2]

[1]*Section for Biotechnology, Department of Chemistry and Bioscience, Aalborg University, Aalborg, Denmark,* [2]*Centre for Quantitative Genetics and Genomics, Aarhus University, Tjele, Denmark.*

Precision medicine has been forecasted to change modern healthcare aiming to provide treatment options targeted towards the patient's genomic profile. During the last decade an enormous effort has been in developing disease specific genetic risk scores (GRS). Until recently, GRS was constructed using information on the disease itself; however, as the human genome has abundant pleiotropy the accuracy of risk stratification may be improved by constructing multi-trait (MT) GRS.

This study investigated whether leveraging correlated information when constructing GRS elevate the predictive accuracy compared with single-trait (ST) GRS. We constructed ST- and MT-GRS for seven common diseases in the UK Biobank using a weighted (by selection index) MT-SBLUP genetic predictor. For each disease the correlated information was obtained from the other six diseases and/or body weight, BMI, smoking status and overall medication-use.

In summary, in four of seven diseases MT-GRS increased the disease prediction accuracy considerable. These results clearly demonstrate the benefit of incorporating correlated information. The added benefit of including correlated information largely depends on the number of observations, type of trait and degree of pleiotropy. In particular, incorporating accurate, easy obtainable information – like BMI and medication-use – seems as an untapped resource.

Disease	No. cases (% of all)[1]	Heritability[2]	Best model			%-improved performance (by model)[3]
			LD pruning (r^2)	*P*-value threshold	Nagelkerke R^2	
Allergic rhinitis	22,116 (7%)	0.20	0.9	0.05	0.012	25% (MT-dis)
Asthma	45,154 (13%)	0.20	0.9	0.05	0.028	-12% (ST)
CAD	25,998 (8%)	0.12	0.9	0.20	0.022	14% (MT-all)
Diabetes T2	18,809 (6%)	0.28	0.1	0.999	0.036	16% (MT-quant)
Hyperlipidemia	35,097 (10%)	0.15	0.9	0.999	0.21	20% (MT-all)
Hypertension	112,213 (33%)	0.20	0.9	0.999	0.048	-5% (ST)
Osteoarthritis	59,833 (18%)	0.17	0.5	0.999	0.014	-5% (ST)

1: Among unrelated, white-British individuals (n = 335,744). 2: LDSC estimates converted to the liability scale using disease prevalence from UK. 3: %-increased in variance explained from single-trait to multi-trait GRS. ST: single trait model, MT-dis: multi-trait by other diseases, MT-quant: multi-trait by quantitative traits, MT-all: diseases and quantitative traits

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P18.022.D Gene set enrichment analysis of basal and in vitro irradiation gene expression differentiates breast cancer patients with late skin radiotherapy toxicity

Ester Aguado-Flor[1], *María J. Fuentes-Raspall*[2], *Ricardo Gonzalo*[3], *Carmen Alonso*[4], *Teresa Ramón y Cajal*[4], *David Fisas*[4], *Alejandro Seoane*[5], *Álex Sánchez Pla*[3], *Jordi Giralt*[6], *Orland Díez*[1,7], *Sara Guitérrez-Enríquez*[1]

[1]*Hereditary Cancer Genetics Group, Vall Hebron Institute of Oncology (VHIO),Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spain,* [2]*Radiation Oncology Department, Santa Creu i Sant Pau Hospital, Barcelona, Spain,* [3]*Statistics and Bioinformatics Unit, Vall d'Hebron Institut de Recerca (VHIR), Vall d'Hebron Hospital Universitari, Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spain,* [4]*Medical Oncology Department, Santa Creu i Sant Pau Hospital, Barcelona, Spain,* [5]*Medical Physics Department, Vall d'Hebron Hospital Universitari, Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spain,* [6]*Radiation Oncology Department, Vall d'Hebron Hospital Universitari, Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spain,* [7]*Area of Clinical and Molecular Genetics Vall d'Hebron Hospital Universitari, Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spain.*

Radiotherapy-induced late effects are determined in part by genetic susceptibility and are a common cause of morbidity amongst cancer survivors. The purpose of this study was to elucidate the molecular basis underlying the radiotherapy-induced late skin toxicity in breast cancer patients. Peripheral blood mononuclear blood cells of 10 patients with severe late complications from radiotherapy and 10 patients without symptoms were mock-irradiated or irradiated with 8-Gy. The 48-h response was analysed by gene expression profiling with Affymetrix Human Exon 1.0 ST arrays. Irradiated and non-irradiated gene expression profiles were compared between both groups of patients. Gene set enrichment analysis (GSEA) was performed to identify the biological pathways associated with the expressed genes. Regardless of patient toxicity status, the 8-Gy irradiation leads to a significant gene expression signature. Although the group of differentially expressed mRNAs did not reach a significant adjusted p-value between patients with or without clinical toxicity, the discriminative power was enhanced by using GSEA approach. Thus, in basal conditions, the differentially expressed genes were mainly involved in transcription and interferon signalling pathways. In contrast, after 8Gy the genes were enriched in cell cycle and G protein-coupled receptor signalling process. Posterior qPCR analysis revealed that *APOBEC3H* (a ssDNA deoxycytidine deaminase with antiretroviral activity) was significantly overexpressed after 8Gy in the non-toxicity patients. In conclusion, the functional profile retrieved from GSEA indicates that immune and DNA biological signatures are associated with radiotherapy-induced late toxicity. Carlos III Institute funded by FEDER-a way to build Europe- [PI05/2181]; ERAPerMed JTC2018 (ERAPERMED2018-244, SLT011/18/00005).

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P18.023.A Unmasking a case of Hereditary Angioedema without C1-INH deficiency in a misdiagnosed type I patient

Alejandro Mendoza-Alvarez[1], *Adrian Muñoz-Barrera*[2], *Itahisa Marcelino-Rodriguez*[1], *Eva Tosco-Herrera*[1], *Beatriz Guillen-Guio*[1], *Almudena Corrales*[1], *Antonio Iñigo-Campos*[2], *Ariel Callero*[3], *Rafaela González-Montelongo*[2], *Jose Miguel Lorenzo-Salazar*[2], *Carlos Flores*[1,2,4]

[1]*Research Unit, Hospital Universitario Nuestra Señora de Candelaria, Santa Cruz de Tenerife, Spain,* [2]*Genomics Division, Instituto Tecnológico y de Energías Renovables (ITER), Santa Cruz de Tenerife, Spain,* [3]*Deparment of Allergy, Hospital Universitario Nuestra Señora de Candelaria, Santa Cruz de Tenerife, Spain,* [4]*CIBER de Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Spain.*

Introduction: Hereditary angioedema (HAE) is a rare disease caused by C1 inhibitor (C1-INH) deficiency or dysfunction or dysregulation of the kinin cascade (HAE-nC1-INH). Despite HAE management guidelines recommend relying on genetic tests, most HAE patients continue to be diagnosed based on protein serum levels. Here, we describe the genetic analysis of an HAE patient from an affected Spanish family who was misdiagnosed as a type I patient.

Material and methods: Biochemical determination of protein levels and activity were carried out. Whole-exome sequencing (WES) data were obtained from six individuals belonging to the same family. Results were analyzed with the Hereditary Angioedema Database Annotation (HADA) tool for causal variant prioritization.

Results: The index patient was a 25-year female with facial and cutaneous attacks, which worsened after the administration of oral contraceptives. Diagnosis based on biochemical analysis supported an HAE type I patient (C4 = 11.3 mg/dl; C1-INH = 17 mg/dl; C1-INH activity = 69%). However, WES detected the *F12* variant affecting function c.983G>T (p.Thr328Lys) in the index and her asymptomatic father. This variant is frequently reported in HAE-nC1-INH patients and is considered pathogenic under ACMG classification guidelines.

Conclusion: Genetic analysis changed the diagnosis of an HAE patient provided by biochemical assays. Variant prioritization by HADA helped to reach a fast and precise identification of the underlying causes. Funding: Ministerio de Ciencia e Innovación (RTC-2017-6471-1; AEI/FEDER, UE); ITER agreement OA17/008; SEAIC Foundation (18_A01); FIISC (FPIFIIS19/48); ACIISI (TESIS2020010002) co-funded by European Social Fund; Instituto de Salud Carlos III (CD19/00231); ECIT CGIEU0000219140.

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P18.026.D Extracellular vesicle enriched miRNAs as prognostic biomarkers in malignant mesothelioma

Katja Goricar[1], *Marija Holcar*[1], *Nina Mavec*[1], *Viljem Kovac*[2], *Metka Lenassi*[1], *Vita Dolzan*[1]

[1]*Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia,* [2]*Institute of Oncology Ljubljana, Ljubljana, Slovenia.*

Introduction: Malignant mesothelioma (MM) is a rare cancer characterized by poor prognosis and short survival. Extracellular vesicles (EVs) are membrane-bound particles released from cells into various body fluids and their molecular composition reflects the characteristics of the origin cell. Blood EVs or their miRNA cargo might serve as new minimally invasive biomarkers of treatment response. Our aim was thus to evaluate miRNAs enriched in serum EVs as potential prognostic biomarkers in MM patients.

Materials and Methods: We performed a pilot longitudinal study that included 20 MM patients. EVs were isolated from serum samples obtained before and after treatment using ultracentrifugation on 20% sucrose cushion. Expression of EV-enriched miR-103-3p, miR-126-3p and miR-625-3p was quantified using qPCR. Nonparametric tests and survival analysis were used in statistical analysis.

Results: After treatment, expression of miR-625-3p and miR-126-3p increased only in MM patients with poor treatment response ($P = 0.012$ and $P = 0.036$, respectively), while no differences were observed in patients with good response ($P = 0.173$ and $P = 0.374$, respectively). A relative increase in miR-625-3p expression after treatment for more than 3.2% was associated with much shorter progression-free survival (7.5 vs 19.4 months, $P = 0.024$) and overall survival (12.5 vs 49.1 months, $P = 0.043$) of MM patients. Bioinformatic analysis identified 33 miR-625-3p targets that were enriched in eight biological pathways.

Conclusions: EV-enriched miR-625-3p could serve as a prognostic biomarker in MM and could contribute to a more personalized treatment in these patients. Research grants: ARRS L3-8203, L3-2622 and P1-0170.

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P18.027.A miR-29b inhibition in triple negative cells activate apoptosis and autophagy related mechanisms

Cornelia Braicu[1], *Raduly Lajos*[1], *Vlad Morhan*[1], *Zsófi Papi*[2], *Ancuta Jurj*[1], *Oana Zanoaga*[1], *Roxana Cojocneanu*[1], *Alin Moldovan*[1], *Cristina Ciocan*[1], *Ioana Berindan-Neagoe*[1]

[1]*Research Center for Functional Genomics, Biomedicine and Translational Medicine, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania,* [2]*Faculty of Medicine, University of Szeged, Szeged, Hungary.*

Introduction: The lack of receptors in triple-negative breast cancer (TNBC) restricts therapeutic options used in clinical management. MicroRNAs (miRNAs) are small, non-coding transcripts affecting cellular mechanisms by regulating gene expression at post-transcriptional level. The study aims to investigate the therapeutic potential of miRNA inhibitors designed for silencing miR-29b, transcript overexpressed in TNBC and correlated with the overall survival in TNBC.

Materials and methods: As TNBC models were used BT549 and MDA-MB-231 cells. The biological effect of the transient miR-29b inhibition was evaluated at cellular and molecular level.

Results: miR-29b inhibition promoted a reduction of cell proliferation and colony forming ability, along with apoptosis and autophagy assessed by confocal microscopy. At molecular-level miR-29b inhibition caused alteration on miRNA pattern (11 downregulated and 8 overexpressed) assessed using microarray technology on BT549. An important downregulated miRNA is represented by miR-185 a biomarker of therapy response targeting MAPK signalling. Additional qRT-PCR, reveals inhibition of Bcl-2 and *TP53*, along with the overexpression of *TGFβ1*, for both cell lines.

Conclusion: miR-29b modulates the crosstalk between apoptosis and autophagy signalling, in same time were activated the drug resistance mechanism, where an important role is related to TGFβ signalling. Our data suggest that miR-29b inhibition act on multiple mechanisms that regulated cell fate, and therefore may serve as a therapeutic target in TNBC.

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P18.028.B Genetic factors implicated in the response to fingolimod treatment in multiple sclerosis patients: results from a pharmacogenetic meta-analysis

Laura Ferrè[1,2,3], *Elisabetta Mascia*[2], *Ferdinando Clarelli*[2], *Tina Roostaei*[4], *Beatrice Pignolet*[5,6], *David Brassat*[5], *Roland Liblau*[5], *Howard L. Weiner*[7,8], *Philip L. De Jager*[4], *Massimo Filippi*[9,10,3], *Federica Esposito*[1]

[1]*Neurology and Neurorehabilitation Unit, IRCCS San Raffaele Hospital, Milan, Italy,* [2]*Laboratory of Human Genetics of Neurological Disorders, IRCCS San Raffaele Hospital, Milan, Italy,* [3]*Vita-Salute San Raffaele University, Milan, Italy,* [4]*Center for Translational and Computational Neuroimmunology, Department of Neurology, CUMC,*

New York, NY, USA, [5]Centre Hospitalier Universitaire de Toulouse, Toulouse, France, [6]Institut Toulousain des Maladies Infectieuses et Inflammatoires (Infinity), INSERM UMR1291 - CNRS UMR5051 - Université Toulouse III, Toulouse, France, [7]Brigham and Women's Hospital, Boston, MA, USA, [8]Harvard Medical School, Boston, MA, USA, [9]Neurology, Neurorehabilitation and Neurophisiology Unit, IRCCS San Raffaele Hospital, Milan, Italy, [10]Neuroimaging Research Unit, IRCCS San Raffaele Hospital, Milan, Italy.

Introduction: Multiple Sclerosis (MS) is a complex disease with high heterogeneity in terms of clinical presentation and treatment response. Pharmacogenetics can help to develop a more personalized approach and to improve disease management. We report the results of a GWAS on fingolimod-treated relapsing-remitting MS patients.

Methods: We included 4 cohorts of fingolimod-treated MS patients from San Raffaele Hospital, Milan, Italy (OSR1: 246 patients, OSR2: 98 patients), Brigham and Women's Hospital, Boston, USA (USA: 136 patients) and the Centre Hospitalier Universitaire de Toulouse, France (CHUT: 81 patients). We classified treatment response according to the NEDA (no evidence of disease activity) criterion at 2 years and time to first relapse (TFR). We performed a GWAS separately on each cohort and meta-analyzed them using a fixed-effect model.

Results: three genome-wide significant variants were associated with TFR: rs9397818A on chr6 increases the risk of an earlier relapse and has an eQTL effect in whole blood on *TFB1M*, key to mitochondrial gene expression, and *TIAM2*, implicated in endothelial function and cell migration; rs2071572A is a risk allele intronic to synaptotagminV, involved in exocytosis of secretory vesicles, with an eQTL effect in brain cortex; finally the risk allele rs6124768A maps to *CD40* locus and increases its expression according to a public eQTL database. No significant variants were identified in the NEDA analysis.

Conclusions: genetic variants possibly implicated in cell migration, neuronal functions and immune response were associated with response to fingolimod. Functional studies are ongoing. This study was supported by "Fondazione Italiana Sclerosi Multipla" [project 2013/R/13].

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P18.029.C Newborn Screening in Unselected Children Using Genomic Sequencing

Min Jian[1], *Xiaohong Wang*[2], *Yuanyuan Sui*[3], *Mingyan Fang*[1], *Ya Gao*[1], *Ruidong Guo*[1], *Yingping Huang*[2], *Chunhua Liu*[3], *Chenchen Feng*[1], *Yuanning Guan*[2], *Yuxiao Gao*[3], *Zhiwei Wang*[2], *Shuli Li*[4], *Bochen Cheng*[1], *Lina Sun*[2], *Fenghua Cui*[5], *Jia Guo*[1], *Ying Zhan*[6], *Jiayu Chen*[7], *Qian Zhao*[7], *Lingyao Guan*[7], *Haorong Lu*[8], *Chao Nie*[1], *Karsten Kristiansen*[9], *Lennart Hammarström*[1], *Xiaojing Jiang*[3], *Junnian Liu*[2], *Fang Chen*[1]

[1]BGI-Shenzhen, Shenzhen, China, [2]BGI-Qingdao, Qingdao, China, [3]Maternal and child health and family planning service center of Huangdao District, Qingdao, China, [4]Traditional Chinese medical hospital of Huangdao District, Qingdao, China, [5]The affiliated hospital of Qingdao university, Qingdao, China, [6]Qindgao west coast new area central hospital, Qingdao, China, [7]China National GeneBank, BGI-Shenzhen, Shenzhen, China, [8]China National GeneBank, BGI-Shenzhen;Guangdong Provincial Key Laboratory of Genome Read and Write, Shenzhen, China, [9]University of Copenhagen, Copenhagen, Denmark.

Introduction: The aim of this study is to investigate potentially curable or treatable medical conditions in unselected newborns using genomic sequencing (GS).

Materials and Methods: 321 newborns from a cohort of pregnant women from Qingdao, China, underwent high-depth GS with the approval of the ethics committee. 61 Mendelian Diseases, 151 Primary Immunodeficiency Diseases and 5 DPWG recommended Essential pharmacogenetic genes were analyzed.

Results: All 321 newborns carried at least one variant at the five DPGW recommended PGx genes. Codeine and clopidogrel require more attention in giving prescription for 25% and 8% of newborns having a decreased function of CYP2D6 and CYP2C19 enzymes respectively. 121 Mendelian pathogenic or likely pathogenic variants associated with 31 inherited diseases were detected. Three children with compound heterozygous variants at *GJB2* and *PAH* were confirmed by Sanger sequencing. Follow-up of the three families confirmed one child was diagnosed with PKU and two children with *GJB2* variants were scheduled to undergo hearing loss testing every six months after genetic counseling due to the nature of incomplete penetrance of hearing loss. 11 heterozygous pathogenic / likely pathogenic variants in eight PID genes were identified in 11 infants.

Conclusions: Our study is the largest to date using GS to sequence unselected newborns. The results suggest that using GS may be a suitable method for screening newborns for variants in a large number of disease associated genes.This study was supported by Guangdong Provincial Key Laboratory of Genome Read and Write (No. 2017B030301011) and Shenzhen Municipal Government of China (JCY20170817145047361).

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P18.030.D Pharmacogenetics of chemotherapy response in osteosarcoma: a genetic variant in *SLC7A8* is associated with progressive disease

Evelien G. E. Hurkmans[1], *Johanne M. G. Groothuismink*[1], *Hanneke I. Vos*[1], *Winette T. A. van der Graaf*[1], *Uta Flucke*[1], *Yvonne M. H. Versleijen-Jonkers*[1], *Melissa H. S. Roeffen*[1], *Jan B. Koenderink*[1], *Jeroen J. M. W. van den Heuvel*[1], *Bart W. B. Schreuder*[1], *Melanie M. Hagleitner*[1], *Hans Gelderblom*[2], *Anne-Marie Cleton-Jansen*[2], *Judith V. M. G. Bovée*[2], *Eveline S. J. M. de Bont*[3], *Leontien C. M. Kremer*[4], *Johannes Bras*[5], *Huib Caron*[4], *Rachael Windsor*[6], *Jeremy Whelan*[6], *Ana Patiño-García*[7], *Anna González-Neira*[8], *Geoff McCowage*[9], *Sumanth Nagabushan*[9], *Federica Saletta*[9], *Daniel Catchpoole*[9], *Henk-Jan Guchelaar*[2], *Han G. Brunner*[1], *D. Maroeska W. M. te Loo*[1], *Marieke J. H. Coenen*[1]

[1]*Radboud university medical center, Nijmegen, Netherlands,* [2]*Leiden University Medical Center, Leiden, Netherlands,* [3]*University Medical Center Groningen, Groningen, Netherlands,* [4]*Emma Children's Hospital/Academic Medical Center, Amsterdam, Netherlands,* [5]*Academic Medical Center, Amsterdam, Netherlands,* [6]*University College Hospital, London, United Kingdom,* [7]*University Clinic of Navarra, Pamplona, Spain,* [8]*Spanish National Cancer Research Center, Madrid, Spain,* [9]*The Children's Hospital at Westmead, Westmead, Australia.*

Introduction: Despite (neo)adjuvant chemotherapy with cisplatin, doxorubicin and methotrexate in primary osteosarcoma, some patients progress during first-line systemic treatment and have a poor prognosis. In this study, we investigated whether patients with progressive disease, have a distinctive pharmacogenetic profile.

Methods: Germline DNA from 287 Dutch high-grade osteosarcoma patients was genotyped using the DMET Plus array (containing 1,936 genetic markers in 231 drug metabolism and transporter genes). Associations between genetic variants and progressive disease were assessed using logistic regression models and associated variants ($P < 0.05$) were validated in independent cohorts of 146 (from Spain and UK) and 28 patients (from Australia). The functional relevance of the most important hits were explored in an immunohistochemistry (IHC) staining and an in vitro HEK293 overexpression model.

Results: In the association analyses of genetic variants and progressive disease, *SLC7A8* rs1884545 and *SLC7A8* rs8013529 were significantly associated with progressive disease and were independently validated in the validation cohorts (meta-analysis: OR 0.22 [0.07-0.63], $P = 0.005$ and OR 0.19 [0.06-0.55], $P = 0.002$, resp.). *SLC7A8* encodes for the L-type amino acid transporter 2 (LAT2) and LAT2 immunohistochemistry of osteosarcoma tissue suggested improved prognosis for patients with higher LAT2 expression ($p = 0.082$). The in vitro LAT2 overexpression model showed no transport inhibition by cisplatin, doxorubicin or methotrexate, however substrate experiments are still ongoing.

Conclusion: This study identified two genetic variants in *SLC7A8* to be associated with progressive osteosarcoma. These results will provide new evidence that could give opportunities to improve treatment of osteosarcoma patients.

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P18.032.B Gene co-expression network analysis of blood-derived transcriptomic data from Parkinson's disease patients implicates immune responses in disease progression

Ana-Luisa Gil-Martinez[1,2], *Aine Fairbrother-Browne*[1,2,3], *John Hardy*[1], *Juan-Antonio Botia*[1,4], *Mina Ryten*[1,2,5]

[1]*Institute of Neurology, University College London, London, United Kingdom,* [2]*Genetics and Genomic Medicine, Great Ormond Street Institute of Child Health, University College London, London, United Kingdom,* [3]*Department of Medical and Molecular Genetics, School of Basic and Medical Biosciences, King's College London, London, United Kingdom,* [4]*Departamento de Ingeniería de la Información y la Comunicaciones, Universidad de Murcia, Murcia, Spain,* [5]*NIHR Great Ormond Street Hospital Biomedical Research Centre, University College London, London, United Kingdom.*

Introduction: Parkinson's disease (PD) is the second most prevalent age-related neurodegenerative disorder worldwide. In its most common, sporadic form, it is characterised by progressive symptoms, which include dementia. However, progression in Parkinson's disease (PD) is heterogeneous suggesting the existence of genes and pathways specifically involved in this process, which could be novel therapeutic targets. In this study, we aimed to identify such genes by analysing data released by the Parkinson's Progression Markers Initiative (PPMI), a longitudinal observational study with 1,610 case-control participants.

Material and Methods: We used transcriptomic data from the PPMI database, which was generated from 4,690 longitudinal blood samples from 1,610 case/control patients. Gene-level expression data was accessed from the AMP-PD portal and the CoExpNets package was used to generate a co-expression network.

Results: The resulting gene co-expression modules were annotated using the WGCNA R package and gprofiler2 to identify modules enriched for cell type-specific markers and biological functions of interest as defined by the Gene Ontology and KEGG databases. Using this approach, we identified two modules of interest, "red" and "turquoise", whose expression (as summarised by the module eigengene) was significantly correlated with case/control status. Both modules, together containing 5,755 genes, were significantly enriched for terms relating to immune responses including "myeloid leukocyte activation", "neutrophil activation" and "adaptive immune response".

Conclusions: These findings are consistent with the growing evidence implicating inflammatory responses in the aetiology and progression of PD.

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P18.033.C Enrollment engagement strategies for a preemptive genomic screen

Michelle Marie Moore, *Alexander van Gerrevink, Bethany Tucker, Murat Sincan, Catherine Hajek*

Sanford Health, Sioux Falls, SD, USA.

Introduction: Preemptive genomic screening can further personalize medical management and care. Engaging enough patients to implement these initiatives can be difficult. Enrollment rates for our health system's preemptive genomic screening program vary from 2% for patient portal invites to greater than 50% with in-person engagement in the cardiology department.

Background: In September 2017, Sanford health launched a preemptive genomic screening program by inviting a small cohort of patients already enrolled in our Biobank to participate without payment, resulting in a 37% enrollment rate. The clinical, public launch had a much lower uptake of approximately 2%. Eligible military veterans received mailed flyers, online invitations, and a coupon code for gratis participation, resulting in an enrollment rate of approximately 8%. In-person enrollment in a cardiology clinic initially had enrollment rates greater than 50%, but this number dropped to 30% after changes in the enrollment process increased enrollment time demands.

Conclusions: In-person engagement has the highest patient enrollment, but this option is often difficult to implement broadly due to the time and resource demands. The increased uptake of the veterans and the initial biobank cohort could indicate that cost is a contributing factor. The actual reason may not be cost, but the perceptual difference between selling the genomic screen versus presenting it as an option to improve patient care. The development of algorithms to predict which patients are most likely to participate may further improve overall engagement in this genomic screen.

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P18.034.D Comprehensive analysis of actionable pharmacogenes based on mining of large-scale data from the Saudi population

Dorota Monies[1,2], *Ewa Goljan*[1,2], *Mohamed Abouelhoda*[1,2], *Brian Meyer*[1,2]

[1]*KFSHRC, Riyadh, Saudi Arabia,* [2]*Saudi Human Genome Program, King Abdulaziz City for Science and Technology, Riyadh, Saudi Arabia.*

Introduction: It is well documented that drug responses are related to Absorption, Distribution, Metabolism and Excretion (ADME) characteristics of individual patients. Many studies have identified genetic variability in many pharmacogenes that are either directly responsible for or are associated with ADME, giving rise to the performance of personalized medicine. Our objective was to provide a comprehensive overview of pharmacogenetic variations in the Saudi population.

Materials and methods: We undertook Next Generation Sequencing data mining of 13,817 unrelated Saudi nationals to identify functional SNP variants in 8 clinically relevant pharamcogenes recommended by the Clinical Pharmacogenetics Implementation Consortium (CPIC). We used Stargazer (bioinformatics tool) to enable complex analysis of NGS based pharmacogenetics data.

Results and conclusion: We identified 59 alleles in 8 pharmacogenes (*CYP2C9, CYP2C19, CYP3A5, CYP4F2, VCORC1, DPYD, TPMT* and *NUDT15*). Functional consequences of pharmacogenetic haplotypes were found to be prevalent especially in CYP genes (with the exception of *CYP3A5*); 10%-44.4% of variants were predicted to be inactive or had decreased activity. In *CYP3A5* we found the highest number (87.5%) of inactive alleles. Only 1.5%, 0.7% and 0.1% of *NUDT15*, *TMPT* and *DPYD* variants, respectively, were predicted to affect gene activity. In contrast, *VKORC1* was found to be functionally, highly polymorphic with 53.7% of Saudi individuals harbour variants that are predicted to result in decreased activity and 31.3% of the population having variants leading to increased metabolic activity. Based upon this study, 99.8% of individuals carry at least one actionable pharmacogenetic variant.

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P18.035.A Detection of relevant pharmacogenetic information through exome sequencing in oncology

simon verdez[1,2], *Juliette Albuisson*[3,4], *Yannis Duffourd*[1,2], *Romain Boidot*[3,4,5], *Manon Reda*[3,5,6], *Christel Thauvin-Robinet*[2,4,7], *Jean-David Fumet*[6], *Sylvain Ladoire*[6], *Sophie Nambot*[2,7], *Maxime Luu*[8], *Patrick Callier*[1,2], *Laurence Faivre*[2,4,7], *Francois Ghiringhelli*[3,4,5,6], *Nicolas Picard*[9]

[1]*UF Innovation en diagnostic génomique des maladies rares, Dijon, France,* [2]*UMR1231 GAD, Inserm - Université Bourgogne-Franche Comté, Dijon, France,* [3]*Platform of Transfer in Cancer Biology, Georges François Leclerc Cancer Center - UNICANCER, Dijon, France,* [4]*Genomic and Immunotherapy Medical Institute, Dijon, France,* [5]*Department of Tumour Biology and Pathology, Georges François Leclerc Cancer Center - UNICANCER, Dijon, France,* [6]*Department of Medical Oncology, Georges François Leclerc Cancer Center - UNICANCER, Dijon, France,* [7]*Centre de Référence maladies rares « Anomalies du Développement et syndromes malformatifs », Centre de Génétique, FHU-TRANSLAD, CHU Dijon Bourgogne, Dijon, France,* [8]*Centre d'Investigation Clinique, module Epidémiologie Clinique/ Essais cliniques, CHU Dijon Bourgogne, Dijon, France,* [9]*Inserm U1248, service de pharmacologie et toxicologie, université de Limoges, CHU de Limoges, F-87042, Limoges, France.*

Introduction: Pangenomic sequencing plays an important role in cancer treatment, and has the potential to reveal germline genomic variations with therapeutic impact. Variant alleles in pharmacogenes are responsible for adverse drug reactions in relation with chemotherapy, antiemetic or pain treatments.

Material and Methods: To evaluate the interest of such pharmacogenetic information, we applied a dedicated pipeline to identify relevant alleles among a list of 67 variants. We extracted these variants from the genomic data of a cohort of 445 solid cancer patients who previously benefited from exome sequencing (ES) for therapeutic issues. After clinical history and bioinformatic analyses, we retained 2 genes known to have an impact on cancer therapy, namely *DYPD* (dihydropyrimidine dehydrogenase gene) and *CYP2D6*. We retrospectively analysed drug plasma concentrations and treatment outcomes in patients bearing at least one variant allele with high clinical relevance based on PharmGKB resources.

Results: Six patients treated with 5-fluorouracil carrying one level 1A PharmGKB variant in *DPYD* showed a decrease in drug mean clearance over the follow-up period ($p < 0.05$). The proportion of patients with vomiting episodes post-chemotherapy differs between ultra-metabolisers for *CYP2D6* and normal metabolisers (40% vs 16%). All patients ($n = 5$) with poor or ultra-metabolisers status presented adverse drug reactions in relation with opioid therapy.

Conclusion: In patients with solid tumor, pangenomic germline sequencing can provide relevant information about common pharmacogenetic alleles likely to be useful to guide therapeutic drug decision as described in this study for drugs interacting with the CYP2D6 and DPD enzymes.

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P18.036.B Understanding of pharmacogenomic testing, adverse drug reactions, and implementation barriers

Bernard Esquivel, ***Ghada Elnashar****, Ellie Jhun, Jessica Savieo, Elimear O'Mahony, Victor Tam, Kurt Wiersma, Julie England*

OneOme, Minneapolis, MN, USA.

Introduction: Life-threatening adverse drug reactions (ADRs) pose a significant health care burden. A study focused on Stevens-Johnson Syndrome (SJS), Toxic Epidermal Necrolysis (TEN), and SJS/TEN showed a mean hospitalization cost of $128 million/year.3 When compared to an average hospital admission, costs were found to be 5-fold higher for aforementioned conditions.3 Our goal was to assess understanding of pharmacogenomic testing, life-threatening ADRs and potential barriers.

Materials and Methods: A survey of 11 questions was created on Survey Monkey and distributed via LinkedIn (duration: 5 days). Questions covered pharmacogenomic knowledge, life-threatening ADRs (specific to SJS (abacavir and HLA-B*57:01; carbamazepine and HLA-B*15:02)), and perspectives on pharmacogenomic implementation barriers. A retrospective analysis of 13994 de-identified patients that ordered OneOme's RightMed Test during 2019-2020 was conducted in order to identify HLA-B risk allele frequency.

Results:A total of 29 survey responses were received. 55% of respondents indicated the main barrier to implementing pharmacogenomics is price and reimbursement. The retrospective analysis showed a frequency of patients positive for HLA-B*57:01 and HLA-B*15:02 alleles to be 6% and 0.8%, respectively.

Conclusion: Pharmacogenomic testing may decrease health care burden by identifying patients with risk variants that may lead to potential life-threatening ADRs. In our assessment, the biggest barrier to implementation is pricing and reimbursement. Up to 6% of the risk variant frequency was observed in our cohort, highlighting the prevalence of high-cost ADRs. More studies are needed to understand the impact on total cost of care.

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P18.037.C Genetic profiling to inform therapeutic decisions in primary care, A qualitative meta-synthesis of barriers and enablers

Sadaf Qureshi[1], *Laura Condon*[2], *Ralph K. Akyea*[2], *Joe Kai*[2], *Nadeem Qureshi*[2]

[1]*NHS Derby & Derbyshire Clinical Commissioning Group, Derby, United Kingdom,* [2]*Division of Primary Care, Nottingham, United Kingdom.*

Introduction: Pharmacogenomic tests are available to guide treatment. In the UK, there are plans to introduce pharmacogenomic panels into primary care. The purpose of this systematic review was to explore what factors are preventing pharmacogenomics being implemented in primary care, and what factors may overcome these barriers.

Materials and Methods: MEDLINE, EMBASE, PsycINFO and CINAHL databases were searched through to July 2020 for studies that reported primary qualitative data of primary care clinicians and patient views. The Critical Appraisal Skills Programme criteria for quality appraisal was undertaken. Data was then extracted thematically and synthesised to uncover descriptive themes and to generate analytical themes related to barriers and enablers to primary care implementation.

Results: From 1659 citations, 17 eligible studies identified across 7 countries, with a sample size of 440 participants comprising of both primary care clinicians and patients views were included in the thematic synthesis. There were 119 barriers that were classified across 7 themes including lack of pharmacogenomic knowledge or awareness, cost of pharmacogenomic testing and confidentiality, privacy and employment discrimination issues. Further "enablers" that would facilitate implementation into practice included recognition of the potential to reduce adverse drug reactions, improve patient motivation and, from the patients' perspective, alignment with a general interest in genetic testing.

Conclusions: the review highlights several relevant barriers to the application of pharmacogenomics in primary care, as well as factors that would facilitate the **Introduction:** These should be considered before introducing a pharmacogenomic panel in primary care. Funding: NIHR School of Primary Care Research

S. Qureshi: None. **L. Condon:** None. **R.K. Akyea:** None. **J. Kai:** None. **N. Qureshi:** None.

P18.038.D Plant extracts as anti inflammatory alternatives at the gene expression level of cytokines

AZIZ ALKHADDOUR

Southern Federal University, ROSTOV ON DON, Russian Federation.

Introduction: This paper explores the impact of phytochemical compounds found in various amounts in pomegranate, grape seeds and garlic extracts on the levels of gene expression of inflammatory cytokines (IL1b, IL6, IL10) and the variation of this reaction to the genotypes of polymorphism of these cytokines and the relation of this effect to concentration of free radicals.

Material and methods: Culture of human peripheral blood leukocytes was used. Pomegranate extract (1.2, 2.4 %), garlic (0.5, 1.2 %), grape seeds (1.2, 2.4 %) were used. Chemiluminescence was used to detect fast flash values and by using real time PCR was detected ct values. Cytokine gene polymorphisms were analyzed using allele-specific PCR.

Results: Pomegranate extract (2.4%) reduces the levels of IL1b gene transcription by 16 times relative to control. There is also a significant decrease in the expression of the IL6 gene compared to the control after the addition of grape seed extract (1.2%) by 100 times. This influence of IL10 gene polymorphism is more pronounced in people with the CC genotype. In parallel, by the levels of gene expression (IL10), the anti-inflammatory function of grape seeds (1.2%) extract is increased. Finally, with the effect of grape seed extract, we have seen elevated free radical concentrations improve interleukin (IL10) gene expression levels.

Conclusion. The phytochemical compounds in pomegranate and grape seed extracts play the role of anti-inflammatory through their effect by decreasing the gene expression of (IL1b, IL6) and increasing (IL10).

A. Alkhaddour: None.

P18.039.A The GOALL and SENSE of clinical implementation of high-throughput genotyping arrays

Jeroen van Rooij, *Annemieke Verkerk, Bahar Sedaghati-khayat, Linda Broer, Jard de Vries, Gaby van Dijk, Joyce van Meurs, Andre Uitterlinden*

Erasmus Medical Centre, Rotterdam, Netherlands.

Introduction: Genetic testing is increasingly used in clinical practice. Genotyping arrays cover a large portion of clinically relevant genetic variation and provide a cost-effective (30 euro per sample), high-throughput standardized alternative to measure many tests in a single assay. In the GOALL project, we pilot specific clinical utilities and applications of arrays at the Erasmus academical hospital. In the SENSE multidisciplinary consortium, we investigate implementation of genetic predictions in various clinical and societal settings. Both efforts extensively collaborate with national and international efforts.

Methods: We investigate the clinical utility of genotyping arrays by: 1) technical validation of common and rare variants 2) offering polygenic risk scores (PRS) as primary utility in clinical practice, 3) prospectively counseling and eligibility of feeding back secondary findings (pharmacogenomics, ACMG mutations) using arrays. Furthermore, we develop a framework needed for FAIR and ethical implementation.

Results: Preliminary results suggest that >90% of clinical genetic variants can be determined by arrays. Comparison of array *vs*. WES of 197 samples shows an non-reference concordance of 95% for singletons. Population studies demonstrate significant case stratification by PRS for breast cancer, coronary artery disease, major depression, osteoarthritis and age-related macular degeneration. Preliminary investigation shows that 95% of all patients carry medically relevant pharmacogenetic variants.

Discussion: Our preliminary results suggest that a portion of genetic testing can cost-effectively be performed by array-based genotyping. In addition, array-based genotyping allows additional reporting of PRSs and pharmacogenomics. Various pilots are ongoing on the return of these results to patients or citizens.

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P18.040.B Improving the Polygenic Score (PGS) Catalog: updates to submissions, ancestry representation, and score harmonization

Samuel Lambert[1,2,3,4], *Laurent Gil*[1,2,5], *Aoife McMahon*[3], *Emily Tinsley*[3], *Shirin Saverimuttu*[3], *Richard Houghton*[1,2,5], *Michael Chapman*[1,2,5], *Laura W. Harris*[3], *Jacqueline A. L. MacArthur*[3], *John Danesh*[1,2,5,6], *Helen Parkinson*[3,2], *Michael Inouye*[1,2,6,4,7]

[1]*University of Cambridge, Cambridge, United Kingdom,* [2]*Health Data Research UK - Cambridge, Cambridge, United Kingdom,* [3]*European Molecular Biology Laboratory, European Bioinformatics Institute, Wellcome Genome Campus, Hinxton, United Kingdom,* [4]*Cambridge Baker Systems Genomics Initiative, Baker Heart and Diabetes Institute, Melbourne, Australia,* [5]*Wellcome Sanger Institute, Wellcome Genomics Campus, Hinxton, United Kingdom,* [6]*National Institute for Health Research Cambridge Biomedical Research Centre at the Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom,* [7]*The Alan Turing Institute, London, United Kingdom.*

The use of polygenic [risk] scores (PGS) for research and clinical applications is often hindered by incomplete reporting and sharing of scores, as well as heterogeneous evaluation and performance in populations of non-European ancestry. To overcome these challenges we developed the PGS Catalog (www.PGSCatalog.org), an open resource of published PGS (including variants: alleles and weights) and consistently curated metadata. The PGS Catalog currently contains >720 published PGS for >190 traits.

The PGS Catalog is accessible through our website, API and FTP, providing a platform for PGS dissemination, research, and translation. Here, we describe novel features to improve the Catalog:

-Users can directly submit data for inclusion in the Catalog. Submitters have the option to embargo pre-publication results until publication.

-PGS that employ multi-ancestry development samples or have been evaluated in different ancestry populations are now easily identifiable through improvements to the handling and display of participant ancestry within each stage of PGS studies (GWAS, development, evaluation). We also show that European ancestry participants are more overrepresented in PGS studies than what has been previously observed for GWAS, and outline future systematic curation efforts.

-Variants in PGS are often heterogeneously described, lacking chromosomal positions and non-effect alleles necessary to re-calculate the score. We describe an adapted pipeline to distribute harmonized versions of PGS in the GRCh37 and 38 genome builds using data from Ensembl Variation.

We invite PGS submissions and user feedback to ensure the continued development and expansion of the PGS Catalog to meet the community's needs.

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P18.042.D Clinical pharmacogenetic analysis in 5,001 individuals with diagnostic whole exome sequencing data

Javier Lanillos[1], *Marta Carcajona*[2], *Paolo Maietta*[2], *Sara Alvarez*[2], *Cristina Rodriguez-Antona*[1]

[1]*Hereditary Endocrine Cancer Group, Human Cancer Genetics Programme, CNIO, Madrid, Spain,* [2]*NIMgenetics, Madrid, Spain.*

Introduction: Whole exome sequencing (WES) is utilized in routine clinical genetic diagnosis. The technical robustness of repurposing large-scale next generation sequencing data for pharmacogenetics has been demonstrated, supporting the implementation of preemptive pharmacogenetics. However, few studies with limited sample size or limited to specific pharmacogenes have explored the clinical utility of adding clinical pharmacogenetics interpretation to diagnostic WES. **Aim:** We performed a systematic analysis of a large cohort of individuals with diagnostic WES, to provide with global and gene-specific clinical pharmacogenetic utility data, population specific differences and rare loss-of-function variation.

Methods: 809 pharmacogenetic alleles, distributed through 19 genes, defined in a Clinical Pharmacogenetics Implementation Consortium guideline were interrogated in 5,001 individuals with a standard diagnostic WES testing (57% Spain; 27% Colombia; 11% Brazil; 5% other). Analysis included variant retrieval, quality data analysis, genotype to diplotype convertion and pharmacogenetic phenotype classification.

Results: We established that 302 alleles in 11 genes could be used to inform of pharmacogenetic phenotypes that changed drug prescription. Each individual carried in average 2.2 alleles and 93% of the cohort could be informed of at least one actionable pharmacogenetic phenotype. Differences in variant allele frequency were observed among the populations studied and the corresponding gnomAD population for 9.4% of the variants. Regarding novel rare variants, we uncovered 453 in 34 PharmGKB Very Important Pharmacogenes.

Conclusion: We provide with the landscape of preemtive actionable pharmacogenetic information using diagnostic WES data, together with population-specific allele variations.

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P18.043.A Polygenic Risk Prediction Ability of Gender-stratified Coronary Heart Disease

Bahar Sedaghati-khayat[1], *Maxime Bos*[2], *Jingyi Tan*[3], *Joyce B.J. van Meurs*[1,2], *Andre Uitterlinden*[1,2], *Catherine Hajek*[4], *Jerome I. Rotter*[3], *Maryam Kavousi*[2], *Jeroen van Rooij*[1]

[1]*Department of Internal medicine, Rotterdam, Netherlands,* [2]*Department of Epidemiology, Erasmus MC, Rotterdam, Netherlands,* [3]*Institute for Translational Genomics and Population Sciences, The Lundquist Institute at Harbor-UCLA Medical Center, Torrance, CA, USA,* [4]*Department of Internal Medicine, Sanford School of Medicine, University of South Dakota, Vermillion, SD, USA.*

Introduction: Coronary heart disease (CHD) causes 13% of global mortality. Early risk detection could reduce the incidence, morbidity, and mortality of the disease. With an estimated heritability of 40-60%, genetic factors could be suitable for primary prevention. This study evaluated a polygenic risk score (PRS) for the CHD in Rotterdam study cohorts (RS) for implementation in the clinical setting.

Methods: The CHD-PRS was constructed of 177 variants and analyzed in 11,375 participants of the RS. Relative risks of the outer quartiles compared to the middle 50% were determined. Additional analyses are ongoing for various PRS distribution bins, age at onset, the impact of lipid-lowering medication use, and integration of PRS with clinical risk prediction.

Results: The PRS significantly predicted CHD in both women ($OR = 1.28$; $p = 1 \times 10^{-11}$) and men ($OR = 1.28$; $p = 9.7 \times 10^{-14}$). Compared to the middle 50% of the population, the upper quartile had 41% increased risk and the lower quartile had a 24% decreased risk. The PRS significantly predicted the age of onset in women ($\beta = -1.32$; $p = 7.0 \times 10^{-2}$) and men ($\beta = -1.03$; $p = 1.6 \times 10^{-4}$). The results are being compared and meta-analyzed with those in the MESA cohort and the Sanford Health System.

Conclusion: The PRS predicts CHD in both men and women and impacts age at CHD onset. These results suggest that adding PRS to clinical risk evaluation could improve primary prevention, for example by preventively treating individuals at the upper tail of the PRS distribution with lipid-lowering medication. Integration of the PRS into clinical risk prediction is currently ongoing to determine exact thresholds and guidelines.

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P18.044.B Idéfix: Identifying accidental sample mix-ups in biobanks using polygenic scores

Robert Warmerdam[1], *Pauline Lanting*[1], *LifeLines Cohort Study, Patrick Deelen*[1,2], *Lude H. Franke*[1]

[1]*Department of Genetics, University Medical Center Groningen, University of Groningen, Groningen, Netherlands,* [2]*Department of Genetics, University Medical Center Utrecht, Utrecht, Netherlands.*

Identifying sample mix-ups in biobanks is essential to allow the repurposing of genetic data for clinical pharmacogenetics. Pharmacogenetic advice based on the genetic information of another individual is potentially harmful. Existing methods for identifying mix-ups are limited to datasets in which additional omics data (e.g. gene expression) is available. Cohorts lacking such data can only use sex, which can reveal only half of the mix-ups. Here, we describe Idéfix, a method for the identification of accidental sample mix-ups in biobanks using polygenic scores.

In the Lifelines biobank we calculated polygenic scores (PGSs) for 25 traits for 32,786 population-based participants. Idéfix then compares the actual phenotypes to PGSs and uses the relative discordance that is expected for mix-ups, compared to correct samples.

In a simulation, using induced mix-ups, Idéfix reaches an AUC of 0.90 using 25 polygenic scores and sex. This is a substantial improvement over using only sex, which has an AUC of 0.75. Idéfix therefore is not yet able to identify every sample mix-up. However, this will likely improve soon, with highly powered GWAS summary statistics that will likely become available for more commonly measured traits.

Nevertheless, Idéfix can already be used to identify a high-quality set of participants for whom it is very unlikely that they reflect sample mix-ups, and therefore could be offered a pharmacogenetic passport. For instance, when selecting the 10% of participants for whom predicted phenotypes adhere best to the actually measured phenotypes, we estimate that the proportion of sample mix-ups is reduced 250-fold.

R. Warmerdam: None. **P. Lanting:** None. **P. Deelen:** None. **L.H. Franke:** None.

P18.045.C BGLT3 and BCL11A variants associated with sickle sell disease phenotype in Angolan children

Miguel Brito[1,2], *Mariana Delgadinho*[1], *Catarina Ginete*[1], *Brigida Santos*[2,3]

[1]*H&TRC - Escola Superior de Tecnologia da Saúde de Lisboa, Instituto Politécnico de Lisboa, Lisbon, Portugal,* [2]*Centro de investigação em Saúde de Angola, Luanda, Angola,* [3]*Hospital Pediátrico David Bernardino, Luanda, Angola.*

Introduction: Despite being a monogenic disease, Sickle cell disease (SCD) shows a remarkably high clinical heterogeneity. Understanding this heterogeneity could provide valuable insights for prognostic markers. The aim of this study was to assess the frequency and influence of polymorphisms in BGLT3 and BCL11A genes in SCD severity.

Materials and Methods: 192 SCD Angolan children were selected. A blood sample was used for hematological and biochemical analyses, fetal hemoglobin quantification and sequencing. Genotype was obtained for 5 SNPs: rs4671393, rs11886868, rs1427407, rs7557939, in BCL11A and rs7924684 in BGLT3.

Results: BCL11A variants frequency ranged between 6.2 to 9.4%, and BGLT3 variant was 2.1%. HbF was statistically associated with the SNPs studied in BCL11A. Three SNPs presented significant values in neutrophil count and in gamma chains ratio. The last also influenced by the variant in BGLT3 gene. This variant also associates with HbF levels, although not significantly.

Discussion: We report for the first time a correlation between a polymorphism in BGLT3 gene (rs7924684) and the gamma G and A globin ratio. Alterations in this ratio are normally indicative of a molecular defect at the level of the HbF synthesis. We confirmed in this population the importance of BCL11A variants in SCD phenotype. SCD has a different clinical presentation between populations of different origins. There are several polymorphisms being discovered every day that could explain the HbF variation between different geographic regions. The results emphasize the importance of personalized health care for SCA patients. Funding FCT/Aga Khan (nº330842553) and FCT/MCTES (UIDB/05608/2020 and UIDP/05608/2020)

M. Brito: None. **M. Delgadinho:** None. **C. Ginete:** None. **B. Santos:** None.

P18.047.A PathWAS analysis sheds a new light on the biology of complex traits

Sebastian Marcus May-Wilson, *Erin Macdonald-Dunlop, SCALLOP Consortium, James F. Wilson, Nicola Pirastu*

University of Edinburgh, Edinburgh, United Kingdom.

Rationale: With the aim of understanding complex traits and multifactorial disease, there has been an increasing focus on studying omics alongside genetic data from GWAS. These studies could, however, be potentially limited by examining the effects of individual genes acting in isolation and not in the context of broader biological networks. Incorporating multiple genes, grouped by pathways, has the potential to increase power of discovery while improving our understanding of the underlying biology.

Method: We selected genes from known biological pathways and then created polygenic risk scores (PRS) from available QTL data. The relative contribution of each gene on overall pathway functionality is estimated by fitting a multivariable Mendelian randomisation (MR) using the *QTLs as exposures against a measured protein "end-point" from the SCALLOP consortium. The PRS and MR results are then combined to create an overall pathway PRS, validated in an independent sample. The significant pathway scores were then tested, using PheWAS, against disease traits in UK Biobank.

Results: Our method successfully predicted the end-point protein level in 8 pathways. These pathways are primarily immune response pathways, such as NOD-like receptor signalling and Toll-like receptor signalling. From these results, PheWAS identified numerous associations between these pathways and traits in UK Biobank such as lymphocyte and leukocyte count but also height, weight and lung-function traits.

Conclusion: Pathway scoring offers the prospect of more powerful and holistic analysis of GWAS results, with the potential to discover relevant causal pathways for complex traits.

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P18.049.C Hereditary diseases and hereditary cancer-predisposing syndromes mutations findings in «healthy individuals» whole genome study in Russia

Gaukhar Zobkova[1], *Ekaterina Kuznetsova*[1], *Maria Makarova*[1], *Olesya Sagaydak*[1], *Elena Baranova*[1,2], *Maksim Belenikin*[1]

[1]*LLC Evogen, Moscow, Russian Federation,* [2]*Federal State Budgetary Educational Institution of Further Professional Education "Russian Medical Academy of Continuous Professional Education" of the Ministry of Healthcare of the Russian Federation, Moscow, Russian Federation.*

Introduction: Analyzing whole genome sequencing of nearly healthy individuals allows assessing inherited diseases occurrence frequency in different populations. The present study was aimed to analyze the Russian's population inherited diseases structure.

Materials and methods: The study involved 1195 individuals (625 males), mean age 43. Peripheral blood tests and preliminary questionnaires were taken from each participant.

Results: We identified 265 participants (22,1%) with hereditary diseases carriage, among them 30 were cases with carriage of two or more hereditary diseases (2,5%). The most frequent were mutations in genes: *GJB2* - 16 cases, *DHCR7* - 11, *PAH* - 9, *NEB* - 7, *AIRE* - 6, *ATP7B* - 6, C9 - 6, *DUOX2* - 6, *HADHA* - 6, *MPO* - 6, *CFTR* - 5, *GALT* - 5, *IDUA* - 5, *PKHD1* - 5. Also, Ehlers-Danlos syndrome, kyphoscoliotic type 2 (*FKBP14*) carriage case was found. It was first described in 2018 for the Russian population. Hereditary cancer-predisposing syndromes were found in 31 participants (2,5%), among them 12 individuals in the questionnaire indicated personal or family cancer history. Mutations were found in genes: *BLM* - 8, *ATM* - 4, *NBN* - 3, *PALB2* - 2, *NTHL1* - 2, *MUTYH* - 2, *MITF* - 2, *CHEK2* - 2, *BRCA1* - 1, *BRCA2* - 1, *PMS2* - 1, *RAD51C* - 1, *BARD1* - 1, *SDHC* - 1.

Conclusions: Whole-genome sequencing of nearly healthy individuals could be useful in the early prevention of cancer development and in offspring planning.

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P18.050.D Effect of clinical and biochemical evidence on the success rate in the diagnosis of inherited metabolic diseases using WES

Laura Gort[1,2], *Joan Anton Puig-Butillé*[3,2], *José Luís Villanueva-Cañas*[3], *Frederic Tort*[1,2], *Antonia Ribes*[1,2], *Judit Garcia-Villoria*[1,2]

[1]*Div of Inborn Errors of Metab. Dep Bioch Molec Genet. Hospital Clínic, Barcelona, Spain,* [2]*CIBERER, IDIBAPS, Barcelona, Spain,* [3]*Molecular biology CORE. Hospital Clínic, Barcelona, Spain.*

Introduction: With the implementation of the NGS sequencing, the process of diagnosing inherited metabolic diseases (IMD) has undergone a substantial change. From clinical and biochemical suspicion to genetic diagnosis performed during decades, to starting directly with the molecular study in the face of a clinical suspicion of nowadays. This powerful tool, however, does not always provide a diagnosis if it is not supported by clear clinical and/or biochemical markers. In this study we checked the success rates when reaching the diagnosis, according to the available clinical and/or biochemical data of patients.

Material and methods: We analyzed 205 patients with suspected IMD using Whole Exome sequencing (WES). Data were analyzed using virtual gene panels based on available clinical and biochemical data from patients.

Results: A total of 205 patients were analyzed, clinical data were available in 64% of cases (132/205) and the genetic diagnosis was reached in 34% of the patients (45/132). In 121/205 (59%) of the patients we had clear biochemical markers that indicated a possible IMD, and in these cohort the genetic diagnosis was achieved in 55% (67/121) of cases. A particular mention are the samples of patients referred from the neonatal screening program, in which newborns still don't show symptoms but they present biochemical markers of pathology. We studied 45 cases of this cohort and genetic diagnosis was reached in 71% of newborns.

Conclusions: When using NGS sequencing, clinical symptoms and positive biochemical markers, greatly improves the percentage of genetic diagnosis in patients with suspected IMD.

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P19 Population Genetics and Evolutionary Genetics

P19.001.A Genotype of autosomal dominant polycystic kidney disease using a custom gene panel in Malta

Natalie Ciantar[1], *Emanuel Farrugia*[2], *Jean Calleja Agius*[1], *Christopher Barbara*[3], *Graziella Zahra*[4], *Edith Said*[1]

[1]*University of Malta, Faculty Medicine and Surgery, Anatomy and Cell Biology Department, Msida, Malta,* [2]*Nephrology and General Medicine Division, Department of Medicine, Mater Dei Hospital, Malta, Msida, Malta,* [3]*Pathology Department, Mater Dei Hospital, Malta, Msida, Malta,* [4]*Molcecular Diagnostics Laboratory, Pathology Department, Mater Dei Hospital, Malta, Msida, Malta.*

Introduction: Polycystic Kidney Disease (PKD) is the commonest form of inherited kidney disorder. The disease can be inherited in an autosomal dominant (AD) or autosomal recessive (AR) manner. Autosomal dominant polycystic kidney disease (ADPKD) is characterized by the development of multiple renal cysts causing renal enlargement and end-stage renal disease (ESRD) in 50% of patients by 60 years of age.

Methodology: A total of 49 unrelated patients with clinical features of ADPKD were studied using a customized gene panel for genes associated with polycystic kidney disease (PKD) using next generation sequencing (NGS). The genes studied were PKD1, PKD2, GANAB, DNAJB11, PKHD1 and DZIP1L.

Results: Bioinformatic analysis has identified five different pathogenic variants in fifteen subjects. Two different novel frameshift pathogenic variants and three other previously reported frameshift, nonsense and splicing pathogenic variants were identified. The novel pathogenic variants, c.4651delC (p.Leu1551SerfsTer12) and c.1645dupG (p.Glu549GlyfsTer24) were identified in PKD1 and PKD2 respectively. Other variants of unknown clinical significance have been identified through sequencing.

Conclusion: This study helps to show that a customized gene panel is the method of choice for studying patients with ADPKD and further emphasizes the genetic variability of this condition. Further functional analysis of these novel variants is necessary to understand the mechanism underlying the development of ADPKD in the Maltese population. This research is being funded by the LifeCycle Malta Foundation through the University of Malta Research, Innovation & Development Trust (RIDT) and by the Tertiary Education Scholarship Scheme.

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P19.002.B Community-based countrywide analysis of variants of the lactase-phlorizin hydrolase gene and their pathological correlates in Libya

Ariej Mohammed[1], ***Inas M. Alhudiri***[1], *Hamza Ben Zeglam*[1], *Abdenaser A. Mohamed*[1], *Hafsa Al-Emam*[1], *Halla Elshwekh*[1], *Nabil S. Enattah*[1,2]

[1]*Biotechnology Research Centre, Tripoli, Libyan Arab Jamahiriya,* [2]*University of Tripoli, Tripoli, Libyan Arab Jamahiriya.*

Introduction: Lactose intolerance is the most common genetic enzyme deficiency in humans. It results from a decline in the activity of the lactase-phlorizin hydrolase enzyme in intestinal cells. Several nucleotide polymorphisms (C/T-13910, G/C-14010, T/G-13915, C/G-13907 and T/C-13913) upstream of the lactase-phlorizin hydrolase gene have been associated with lactase persistence in European, African and Middle Eastern populations. The aim was to study the prevalence of lactose-persistence associated variants of the lactase-phlorizin hydrolase gene in Libyan population and its correlation with digestive symptoms.

Methods: Buccal DNA swabs were collected from 242 adults from the western, southern and eastern regions of Libya. A DNA region spanning the C/T-13910 variant was analyzed.

Results: New variants (T/A-13883, A/C-13921, T/C-13961 and C/A-13962) were detected in the analyzed region in addition to the previously described C/T-13910 and T/G-13915 variants. The prevalence of the lactose persistence phenotype was associated with variants C/T-13910 and T/G-13915, which were collectively detected in 30% of the study participants. The allele frequency of the lactose persistence G_13915 variant was 0.133 (SD ± 0.016), whereas the frequency of the T_13910 allele was 0.029 (SD ± 0.008). The most common was the Arab variant T/G-13915 in the eastern region followed by the European C/T-13910 variant. The T/G-13915 variant was associated with the presence of abdominal symptoms ($p = 0.005$).

Conclusions: Both new and well-known variants of the lactase-phlorizin hydrolase gene associated with lactose persistence are common in Libyans. Further studies are needed to confirm the association of the newly discovered variants with lactose persistence.

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P19.003.C Association of archaic introgression tracts to modern human facial features

Pierre Faux[1], *Betty Bonfante*[1], *Javier Mendoza-Revilla*[2,3], *Rolando Gonzalez-José*[4], *Lavinia Schüler-Faccini*[5], *Maria-Catíra Bortolini*[5], *Victor Acuña-Alonzo*[6], *Samuel Canizales-Quinteros*[7], *Carla Gallo*[3], *Giovanni Poletti*[3], *Gabriel Bedoya*[8], *Francisco Rothhammer*[9], *Kaustubh Adhikari*[10,11], *Andres Ruiz-Linares*[1,11,12]

[1]*UMR7268 ADES, Aix-Marseille University, Marseille, France,* [2]*Unit of Human Evolutionary Genetics, Institut Pasteur, Paris, France,* [3]*Laboratorios de Investigación y Desarrollo, Universidad Peruana Cayetano Heredia, Lima, Peru,* [4]*Instituto Patagónico de Ciencias Sociales y Humanas, Centro Nacional Patagónico, CONICET, Puerto Madryn, Argentina,* [5]*Departamento de Genética, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil,* [6]*Molecular Genetics Laboratory, National School of Anthropology and History, Mexico City, Mexico,* [7]*Unidad de Genomica de Poblaciones Aplicada a la Salud, UNAM-Instituto Nacional de Medicina Genómica, Mexico City, Mexico,* [8]*GENMOL, Universidad de Antioquia, Medellín, Colombia,* [9]*Instituto de Alta Investigación, Universidad de Tarapacá, Arica, Chile,* [10]*School of Mathematics and Statistics, The Open University, Milton Keynes, United Kingdom,* [11]*Department of Genetics, Evolution and Environment, and UCL Genetics Institute, University College London, London, United Kingdom,* [12]*Ministry of Education Key Laboratory of Contemporary Anthropology and Collaborative Innovation Center of Genetics and Development, Fudan University, Shanghai, China.*

Introduction: Evidence of the functional legacy of archaic hominids in modern humans is still limited to a few genes and phenotypes. Here, we performed scans of archaic (Neanderthal and Denisovan) introgression in modern humans on regions significantly associated with facial features and tested whether the

(archaic/modern) origin of alleles is significantly associated to these phenotypes or not.

Material and methods: Genotype data from about 6,200 admixed individuals from 5 Latin American countries was imputed using 1000 Genome Phase III data and locally phased at 34 regions significantly associated with facial features. Then, keeping only SNPs with high confidence of sequencing on either Denisovan or Altai Neanderthal samples, we modelled each chromosomal phase of each individual as the most likely sequence of modern (using the 1000 Genome YRI population as the reference for modern human) and archaic segments. At each SNP, we eventually coded each individual genotype as the number of alleles falling into a high-confidence (>99%) archaic tracts and tested that SNP for association with phenotypes.

Results. We found significant associations between the so-coded genotype and various facial features. Our most prominent result involves the TBX15-WARS2 region, in which a 25-Kb tract likely inherited from Denisovans was previously found to be highly frequent in East-Asian and Native Americans populations. We associated that tract with lip thickness ratio in modern humans.

Conclusions: The strategy followed in this study shows potential to understand why archaic tracts remained in modern humans and to predict phenotypic features of archaic humans.

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P19.005.A Population structure and selection analysis from Cameroon next-generation sequencing data

Sonia Olaechea-Lázaro[1], *Neskuts Izagirre*[1], *Saioa López*[2], *Óscar García*[3], *Krishna R. Veeramah*[4], *Garrett Hellenthal*[5], *Mark Thomas*[5], *José Miguel Lorenzo-Salazar*[6], *Carlos Flores*[6,7,8], *Santos Alonso*[1]

[1]*University of the Basque Country, Leioa, Spain,* [2]*Wellcome Trust, London, United Kingdom,* [3]*Ertzaintza, Erandio, Spain,* [4]*Stony Brook University, New York, NY, USA,* [5]*University College London, London, United Kingdom,* [6]*Instituto Tecnológico y de Energías Renovables, Santa Cruz de Tenerife, Spain,* [7]*Instituto de Tecnologías Biomédicas (Universidad de La Laguna), Santa Cruz de Tenerife, Spain,* [8]*Hospital Universitario Nuestra Señora de Candelaria, Santa Cruz de Tenerife, Spain.*

Introduction: Cameroon is considered an "Africa-in-miniature" not only due to its high genetic,ecological, and linguistic diversity, but also due to the wide variety of subsistence strategies adoptedby its inhabitants. Here we assess whole-exome and whole-genome sequencing data to understandhow environmental factors shape human genomic diversity.

Materials and Methods: We analysed 100 whole-exomes and 10 genomes from Cameroonsampling 30 ethnic groups (including Fulani, Bassa, Kotoko, Mambila). We evaluated populationstructure and diversity (PCA, Fst) and signatures of selection (Tajima's D, PBS). Given that buccalswabs were the DNA source and a proportion of the reads were unmapped (~1%), these have beenused to identify the oral microbiome.

Results: Our analysis suggest that Cameroonians might be genetically subdivided into three mainpopulation clusters that locate to the North, West, and coast regions of the country. Moreover, weidentify putative region-specific selection signals associated to environmental factors. For example,our initial results suggest that there is evidence of balancing selection in *TARBP1*, a gene involvedin the development of AIDS.

Conclusions: We identify population structure and signatures of natural selection in the genomes ofCameroonian peoples. Whole-exome sequencing has proved an adequate tool to assess thesephenomena.

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P19.006.B Childhood maltreatment as a modifier of genetic risk for cardiovascular disease: cross-sectional and prospective analysis of UK Biobank

Helena Urquijo[1,2], *Ana L. G. Soares*[1,2], *Abigail Fraser*[1,2], *Laura D. Howe*[1,2], *Alice R. Carter*[1,2]

[1]*MRC Integrative Epidemiology Unit, University of Bristol, Bristol, United Kingdom,* [2]*Population Health Sciences, Bristol Medical School, University of Bristol, Bristol, United Kingdom.*

Rationale: Childhood maltreatment is consistently associated with CVD and may modify genetic susceptibility to adverse cardiovascular phenotypes.

Objective: To investigate whether childhood maltreatment modifies the genetic susceptibility to a range of cardiovascular risk factors and diseases.

Methods and Results: We used genetic and phenotypic data from 100,833 UK Biobank participants. A questionnaire administered in mid-life was used to assess exposure to childhood maltreatment. We regressed nine CVD risk factors and subtypes on their respective polygenic scores (PGS) and exposure to childhood maltreatment using linear and logistic multivariate regression, adjusted for sex, age and 40 genetic principal components. Effect modification by exposure to child maltreatment was tested on the additive and multiplicative scales through the inclusion of a product term (PGS*maltreatment) in the regression models. On the additive scale, childhood maltreatment modified genetic susceptibility to higher BMI, with an increase of 0.099 SD (95% CI: 0.038-0.160) in BMI per unit increase in maltreatment score ($P_{effect\ modification}$: 0.003). On the multiplicative scale, similar results were obtained for BMI though these did not withstand to Bonferroni correction ($P_{effect\ modification}$: 0.015). There was little evidence of effect modification on the other eight cardiovascular traits or of sex-specific effects. Sensitivity analyses adjusting for childhood socioeconomic position and covariate interactions yielded similar results.

Conclusions: Individuals exposed to childhood maltreatment may have an exacerbated genetic susceptibility to a higher BMI. Replication and validation using larger cohorts may clarify whether childhood maltreatment modifies genetic risk for other adverse cardiovascular phenotypes.

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P19.008.D Circadian rhythm gene polymorphisms and susceptibility to metabolic syndrome: a meta-analysis

Ivana Škrlec[1], *Jasminka Talapko*[1], *Snjeana Dijan*[1], *Hrvoje Lepeduš*[2,3]

[1]*Faculty of Dental Medicine and Health, Osijek, Croatia,* [2]*Faculty of Dental Medicine and Health Osijek, Osijek, Croatia,* [3]*Faculty of Humanities and Social Sciences, Osijek, Croatia.*

Introduction: Metabolic syndrome (MetS) is a set of cardiovascular risk factors associated with type 2 diabetes, obesity, and cardiovascular diseases. Research findings of the association between circadian rhythm gene polymorphisms and MetS and its comorbidities are not consistent. This meta-analysis was performed to quantify the relationships between circadian rhythm genes and the risk of MetS.

Materials and Methods: The PubMed and Scopus databases were searched for studies reporting on the association between circadian rhythm gene polymorphisms (*ARNTL, BMAL1, CLOCK, CRY, PER, NPAS2, RORα, REV-ERBα*, and *REV-ERBβ*) and MetS, and its comorbidities type 2 diabetes, obesity, and hypertension. A random-effect model was used to calculate the pooled odds ratio and 95% confidence interval by comprehensive meta-analysis software.

Results: Eleven independent studies were analyzed with 16,431 subjects in total. The meta-analysis revealed a significant association between circadian rhythm gene polymorphisms and MetS (OR = 1.19, 95% CI: 1.04-1.38, p = 0.013). The subgroup analysis on comorbidity related to MetS revealed that type 2 diabetes was associated with circadian rhythm genes (OR = 1.07, 95% CI: 1.00-1.14, p = 0.04). Furthermore, the subgroup analyses revealed that *BMAL1* and *CLOCK* genes were associated with MetS (OR = 1.26, 95% CI: 1.05-1.52, p = 0.014, and OR = 1.49, 95% CI: 1.23-1.80, p < 0.001, respectively) with significant heterogeneity (I^2 = 75.3%, p = 0.001).

Conclusion: This study suggests that circadian rhythm gene polymorphisms might be associated with MetS and its comorbidity and potentially cause cardiovascular diseases. Grant no. IP8-FDMZ-2020

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P19.009.A Clinical consequences of rare variants in cerebral small vessel disease genes in UK Biobank

Amy C. Ferguson[1], *Konrad Rawlik*[1], *Rainer Malik*[2], *Sophie Thrippleton*[1], *David Henshall*[1], *Martin Dichgans*[2], *Albert Tenesa*[1], *Cathie Sudlow*[1], *Kristiina Rannikmae*[1]

[1]*University of Edinburgh, Edinburgh, United Kingdom,* [2]*University of Munich, Munich, Germany.*

Introduction: An emerging minority of cases with cerebral small vessel disease (cSVD) are monogenic, with some cases manifesting additional extra-cerebral phenotypes. Currently, variant penetrance data predominantly comes from small disease cohorts and biased case studies. We investigated this in a large population-based study.

Methods: We identified previously-reported pathogenic rare variants in *COL4A1*, *COL4A2*, *TREX1*, *CTSA* and *HTRA1* and their reported phenotypes, mapping phenotypes to hospital admission and primary care codes. We identified the proportion of pathogenic variant carriers with at least one relevant phenotype, to estimate penetrance among 199,945 exome-sequenced UK Biobank participants. We created phenotype clusters for each gene, assigning participants a phenotype score based on their number of cluster phenotypes. We tested for significant differences in phenotype scores between carriers and non-carriers.

Results: We identified 1,143 carriers (Table). Penetrance across genes ranged from 4% to 25% based on hospital data, and 8% to 74% when including primary care. *COL4A1* carrier-status was significantly associated with a greater hospital-based phenotype score compared to non-carriers (OR 1.053, p 0.007). There were no significant associations between carrier-status and phenotype scores for other genes, or in primary-care subgroup analyses.

Conclusion: Our data suggest incomplete penetrance of pathogenic variants in cSVD genes in a population-based dataset. *COL4A1* carrier-status is associated with a greater phenotype score, highlighting the importance of the wider spectrum of phenotypic manifestations in cSVD.

Grants: MR/S004130/1;RE/18/5/34216

Phenotype cluster	Data sources	Variant carriers		
		With ≥1 cluster phenotype (%)	With no cluster phenotypes (%)	Total number of carriers
TREX1-associated phenotypes[a]	Hospital	11 (17%)	53 (83%)	64
	Primary care	11 (58%)	8 (42%)	19
	Hospital & primary care	11 (58%)	8 (42%)	19
CTSA-associated phenotypes[b]	Hospital	7 (25%)	21 (75%)	28
	Primary care	4 (44%)	5 (56%)	9
	Hospital & primary care	4 (44%)	5 (56%)	9
HTRA1-associated phenotypes[c]	Hospital	25 (11%)	209 (89%)	234
	Primary care	63 (74%)	22 (26%)	85
	Hospital & primary care	63 (74%)	22 (26%)	85
COL4A1-associated phenotypes[d]	Hospital	94 (20%)	388 (80%)	482
	Primary care	80 (48%)	86 (52%)	166
	Hospital & primary care	84 (51%)	82 (49%)	166
COL4A2-associated phenotypes[e]	Hospital	14 (4%)	322 (96%)	336
	Primary care	7 (7%)	93 (93%)	100
	Hospital & primary care	8 (8%)	92 (92%)	100

Number of participants with both exome and hospital admission data = 199,945 Number of participants with both exome and primary care data = 67,764 Number of participants with exome, hospital admission and primary care data = 67,764. a: retinal vasculopathy, nephropathy, anaemia, Raynaud's phenomenon, liver disease, migraine, stroke, and vascular dementia b: hypertension, muscle cramp, dry mouth, migraine, stroke, and vascular

dementia c: hair loss, degenerative spine disease, backpain, anaemia, migraine, stroke, and vascular dementia d: cataracts, anterior segment dysgenesis, kidney cyst, haematuria, muscle cramp, myalgia, arrhythmia, Raynaud's phenomenon, haemolytic anaemia, migraine, stroke, and vascular dementia e: stroke and vascular dementia

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P19.010.B Pharmacogenetic defects in CYP 1, 2 & 3 gene families, detected by NGS in Bulgarian individuals

Svetlana Yovinska, *Kalina Mihova, Kunka Kamenarova, Radka Kaneva, Rumen Nikolov, Ivanka Dimova*

Medical University Sofia, Sofia, Bulgaria.

Introduction: Cytochrome P450 (CYP) superfamily is the major determinant of drug pharmacokinetic and therapeutic responses. Families CYP 1, 2, and 3 are responsible for the biotransformation of most xenobiotics, including 70-80% of all drugs in clinical use. Evidence is constantly accumulating for the clinical significance of these CYPs in terms of adverse reactions, drug efficacy, and drug dose determination. Multiallelic genetic polymorphisms, which are highly dependent on ethnicity, play a major role in the function of CYPs and lead to various pharmacogenetic phenotypes divided into poor, intermediate, extensive, and ultrarapid metabolizers.

Materials and methods: We have collected sequence data from 200 Bulgarian patients, which have been attending our lab for diagnostics, using NGS by TrueSighOne platform. Twenty-two genes belonging to CYP 1, 2 and 3 families were sequenced among others. Using this data, we can determine the type and frequency of pharmacogenetic defects in Bulgarian population with high impact on prediction of adverse drug reactions and health care consequences.

Results and discussion: The most frequent drug response associated polymorphisms were discovered in *CYP2D6* (c.506-1G>A) and *CYP2B6* (c.516G>T), followed by *CYP2D6* (c.100C>T), *CYP2B6* (c.785A>G) and *CYP2C9* (c.1075A>C). Data from our research indicates highest frequency of abnormal variants for metabolizing debrisoquine and methadone in Bulgarians. CYP2D6 polymorphism is most often inherited in an autosomal recessive fashion (two nonfunctional alleles for the CYP2D6 gene). Many other drugs are inefficiently metabolized in these patients, including antidepressants (Doxepin, Trimipramine, Imipramine), and selective estrogen receptor modulator Tamoxifen, used for treatment and prevention of breast cancer.

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P19.011.C Impact of host genetic variation on cytokine response variability upon BCG vaccination in children from Guinea-Bissau

Collins Kwadwo Boahen[1], *Simmone J. C. F. Moorlag*[1], *Kristoffer Jarlov Jensen*[2], *Vasiliki Matzaraki*[1], *Ivan Monteiro*[3], *Charlotte de Bree*[1], *Peter Aaby*[4], *Mihai G. Netea*[1,5], *Christine Stabell Benn*[6], *Vinod Kumar*[1,7]

[1]*Department of Internal Medicine and Radboud Center for Infectious Diseases, Radboud University Medical Center, NIJMEGEN, Netherlands,* [2]*Center for Clinical Research and Prevention, Frederiksberg and Bispebjerg Hospital, Frederiksberg, Denmark,* [3]*Bandim Health Project, Indepth Network,codex 1004, Bissau, Guinea-Bissau,* [4]*Bandim Health Project, Statens Serum Institut, Copenhagen S, Denmark,* [5]*Department for Genomics & Immunoregulation, Life and Medical Sciences Institute,University of Bonn, Germany, Bonn, Germany,* [6]*Danish Institute for Advanced Study, University of Southern Denmark, Odense, Denmark,* [7]*University of Groningen, University Medical Center Groningen, Department of Genetics, Groningen, Netherlands.*

Introduction: Over 25% of tuberculosis (TB) deaths occur in the African Continent. Bacillus Calmette-Guerín (BCG) being TB vaccine also provides non-specific protective effects against other infections through "trained innate immunity". However, which genetic mechanisms modulate cytokine responses upon BCG vaccination and how they vary between Africans and Europeans are unknown.

Materials and Methods: An African cohort (Guinea-Bissau) of low-birth-weight (<2.5 kg) infants (~500 samples) was randomized to BCG vaccination or no BCG-vaccination. In vitro stimulation of whole blood using five different stimuli was followed by seven different cytokine measurements. We performed genome-wide SNP cytokine QTL (cQTL) mapping followed by pathway enrichment and functional annotation. The results were compared using a European BCG adult cohort ($n = 300$).

Results: We identified 9 independent cQTLs ($P < 5 \times 10^{-8}$) affecting cytokine responses specifically in the BCG group; unidentified in the control group. Interestingly, these cQTLs show pleiotropic effects. Only one locus out of 9 showed association in the European BCG cohort. Also, nominal cQTLs ($p < 0.05$) between European and African samples showed very limited overlap (1.4% to 1.5%), indicating either age or ethnicity-associated genetic effects. We identified several causal genes at these loci and implicated complement pathway in regulating cytokine response after BCG vaccination. We demonstrate that SNPs in *C1RL* locus affect topologically associated domain by regulating long non-coding RNA expression, which also affects other co-expressed genes.

Conclusion: Our study shows that distinct genetic loci affect cytokine response in African infants with and without BCG-vaccination as well as in European BCG-vaccinated adults.

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P19.012.D Natural selection analysis for GWAS SNPs in cytokine genes

Maryam B. Khadzhieva[1,2,3], *Dmitry S. Kolobkov*[2,4], *Alesya S. Gracheva*[1,2], *Artem N. Kuzovlev*[1], *Serikbay K. Abilev*[2], *Lyubov E. Salnikova*[1,2]

[1]*Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology, Moscow, Russian Federation,* [2]*Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow, Russian Federation,* [3]*Dmitry Rogachev National Research Center of Pediatric Hematology, Oncology and Immunology, Moscow, Russian Federation,* [4]*Weizmann Institute of Science, Rehovot, Israel.*

Cytokines are proteins and glycoproteins implicated in innate and acquired immunity, embryogenesis, hematopoiesis, inflammation and regeneration processes, and proliferation. We applied natural selection tests to identify GWAS cytokine SNPs under positive selection. We generated a list of human genes encoding proteins with cytokine/chemokine and cytokine/chemokine receptor activity employing the QuickGO database ($n = 314$). A total of 3077 associations for 1760 unique SNPs were found for these genes in the NHGRI-EBI GWAS Catalog. Fst (Fixation index) and iHS (Integrated Haplotype Score) for GWAS SNPs were analyzed with the use of 1000 Genome Selection Browser 1.0 (http://hsb.upf.edu/

). This resource provides statistics on natural selection as the absolute scores and rank scores representing −log10(*P*-value) at 0.01 FDR for the SNP compared to others in the whole-genome context. SNPs with Fst scores ≥0.5 or iHS scores ≥2.0 are considered to be under positive selection. SNPs under selection pressure were more often associated with different types of measurements in comparison with other GWAS SNPs: 85.09% (348/409 associations) vs. 70.27% (1865/2654 associations), $P = 6.9E{-}10$; the most pronounced differences were related to anthropometric measurements ($P = 1.4E{-}10$). A total of 75 SNPs had global Fst rank scores >2 (scores 0.404−0.668). Only ten SNPs had rank scores >2 for the iHS CEU score. Natural selection analysis identified top SNPs in the *GDF5* (confirmatory information) and *IL18R1* (new data) genes subjected to positive selection (table 1).

Table 1. Natural selection statistics for the GWAS SNPs in the GDF5 and IL1RL1/IL18R1 genes

Mapped gene	SNPs	Diseases and traits in GWAS Catalog*	Fst Glob score	Fst Glob, −log10 (*P*-value)	iHS CEU score	iHS CEU, −log10 (*P*-value)
GDF5 (growth differentiation factor 5)	rs143384	anthropometric measurements (BMI, waist-hip ratio, hip circumference and other)	0.554	2.753	----	----
	rs224333	anthropometric measurements (BMI-adjusted waist-hip ratio, physical activity measurement, body height)	0.584	2.923	----	----
IL18R1 (interleukin 18 receptor 1)	rs2001461	blood protein (IL18R1) measurement	----	----	4.544	3.554
	rs6419573	atopic eczema	----	----	3.341	2.382
IL1RL1 (interleukin 1 receptor like 1), *IL18R1* (interleukin 18 receptor 1)	rs1420103	serum ST2 measurement	----	----	3.675	2.405

*GWAS signals for the rs143384 and rs224333 were reported for European and mixed ancestry populations. GWAS signals for the rs2001461, rs1420103 and rs6419573 were reported for European ancestry individuals.

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P19.013.A The gene encoding the viral sensor, RIG-1, contains African-specific regulatory variants

Darisia Moonsamy, *Nikki L. Gentle*

School of Molecular and Cell Biology, University of the Witwatersrand, Johannesburg, South Africa.

Introduction: RIG-1, plays an important role in the detection of RNA viruses. Susceptibility to viral infection and disease progression is known to vary between geographically distinct populations. Despite this, African populations are often underrepresented in immunogenetic studies. We therefore sought to identify and characterise African-specific regulatory variants within *DDX58*, the gene encoding RIG-1.

Materials and Methods: Phased single nucleotide polymorphism data from the Phase 3 release of the 1000 Genomes Project (n = 2504) were analysed using VCFtools v (0.1.16), in order to identify bi-allelic variants within *DDX58* that are unique to African populations. Regulatory variants were then annotated using ANNOVAR v (2018-04-16) to identify those with potential effects on RIG-1 expression, splicing and/or function. Known disease-associated variants were prioritised and the haplotype structure surrounding these variants was characterised.

Results: Our analysis revealed 120 variants across the regulatory regions in and around *DDX58*. Of these, 39 were unique to African populations. The patterns of linkage disequilibrium surrounding these variants were also shown to be distinct between population groups. Variants of interest identified include rs540969727, which lies within an H3K4 methylation site, and the minor allele (A) of which is present at frequencies approaching 2% in African populations.

Conclusions: African populations harbour population-specific genetic variants that may alter the expression of the RNA sensor, RIG-1. The potential effects of these variants on susceptibility to viral infection and/or disease progression warrant further investigation. DM and NG are supported by the National Research Foundation (grant numbers 123456 and 122000)

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P19.014.B NGS survey for rare genetic variants, associated with diabetes mellitus, in Bulgarian individuals

Ivanka Dimova[1], *Kalina Mihova*[1], *Kunka Kamenarova*[1], *Ivelina Mihaleva*[2], *Pavlina Gateva*[2], *Radka Kaneva*[2]

[1]*Molecular Medicine Center, Medical University Sofia, Sofia, Bulgaria,* [2]*Department of Pharmacology, Medical University Sofia, Sofia, Bulgaria.*

Introduction: Advances in sequencing technology enabled focused explorations on the contribution of rare variants (MAF < 1%) to human traits. Rare variants in or near genes display larger effects on phenotype compared with regulatory and common genetic variants. However, population stratification poses unique challenges in studies of rare variants. Systematic differences in allele frequencies due to ancestry are more pronounced for rare variants. In our study we aimed in NGS (Next generation sequencing) searching for rare /pathogenic variants in genes, connected to diabetes mellitus (DM), in Bulgarian individuals.

Materials and methods: The sequencing data from clinical exome sequencing of 100 Bulgarians, mostly under the age of 25 years, was analysed for rare/pathogenic genetic variants in 68 genes, known for its association with DM.

Results: We discovered rare/pathogenic variants in three genes, listed in the Table.

Gene	Function	Gene variant	Frequency
GCGR	glucagon receptor involved in glucose regulation	c.118G>A	6%
ABCC8	instructions for making the sulfonylurea receptor 1 protein in the beta cells of the pancreas	c.2921-9G>A	1%
INSR	Insulin receptor	c.3034G>A	1%

The variant in *GCGR* was detected with a higher frequency of 6% in Bulgarian individuals. The gene encodes receptor for glucagon, which exerts insulin opposite effect on glucose metabolism. There is recent evidence for potential targeting glucagon receptor to improve glycemic control.

Conclusion: We demonstrated population/ethnic difference in the frequency of previously announced rare genetic variants, suggesting higher contribution risk effect of *GCGR* to DM. Genetic detection of DM sub-phenotypes could be useful to personalize screening and care. Acknowledgement: BSNF, Contract NoКП-06-H33/10, 2019.

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P19.015.C Inferring Effective Population Size and Divergence Time in the Lithuanian Population According to High-Density Genotyping Data

Alina Urnikyte[1], *Alma Molyte*[1], *Erinija Pranckevičienė*[1], *Zita Aurelė Kučinskienė*[2], *Vaidutis Kučinskas*[1]

[1]*Department of Human and Medical Genetics, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, Vilnius, Lithuania,* [2]*Department of Physiology, Biochemistry, Microbiology and Laboratory Medicine, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, Vilnius, Lithuania.*

The analysis of geographically specific regions and the characterization of fine-scale patterns of The prehistory of the Lithuanian population and genetic relationship to other populations are poorly studied. Thus, the Lithuanian population, as an object of study, is interesting due to its partial isolation with genetic distinctiveness within the European context and with preserved ancient genetic composition. The main objects of this study was to infer demographic parameters, effective population size (*Ne*), and divergence time using high-density single nucleotide polymorphism (SNP) genotyping data generated with the Illumina HumanOmmiExpress-12v1.1 array in 295 individuals from the Lithuanian population and to compare our data with other populations from the Human Genome Cell Line Diversity Panel (HGDP-CEPH). We also aimed to reconstruct past events between the main ethnolinguistic regions—Aukštaitija and Žemaitija of Lithuania. Historically, these regions probably developed as two independent Baltic tribes. Our results of *Ne* in the Lithuanian population through time demonstrated a substantial reduction of *Ne* over the 150,000–25,000 years before present (YBP). The estimated long-term *Ne* of the Lithuanian population is quite low—it equals 5404, which likely is a consequence of the bottlenecks associated with the last glacial period of 25,000–12,000 YBP in Europe. The obtained divergence time estimates between the study populations are in agreement with recent studies. The reconstructed past events in Aukštaitija and Žemaitija showed significant differences between these two regions of Lithuania. This study is a part of the ANELGEMIA project, which has received funding from the Research Council of Lithuania (LMTLT), agreement No. S-MIP-20-34.

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P19.016.D Evidence of a mediating role of fasting insulin, bioavailable testosterone and sex hormone-binding globulin in the relationship between body mass index and endometrial cancer risk: A Mendelian randomization study

Emma Hazelwood*, James Yarmolinsky, Richard M. Martin*

MRC Integrative Epidemiology Unit, University of Bristol, Bristol, United Kingdom.

Endometrial cancer is the fourth most commonly diagnosed cancer in women in the UK. Elevated body mass index (BMI) is an established risk factor and is estimated to confer a larger effect on endometrial cancer risk than any other cancer site. However, the mechanisms underpinning this association remain unclear. We performed two-sample Mendelian randomization (MR) to evaluate the causal effects of BMI and 14 previously hypothesised molecular risk factors (including fasting insulin (FI), bioavailable testosterone, sex hormone-binding globulin (SHBG)) on endometrial cancer risk (12,906 cases, 108,979 controls). Multivariable MR was used to evaluate and quantify the mediating role of the risk factors in the relationship between BMI and endometrial cancer risk. In MR analysis, BMI (per SD (4.6 kg/m^2) increase: OR = 1.86, 95% CI = 1.60-2.17, $P = 2.68\text{x}10^{-15}$), FI (per natural log transformed pmol/L increase: OR = 3.42, 95% CI = 2.02-5.80, $P = 4.55\text{x}10^{-6}$), bioavailable testosterone (per inverse normal transformed nmol/L increase: OR = 1.47, 95% CI = 1.30-1.66, $P = 3.82\text{x}10^{-10}$) and SHBG (per inverse normal transformed nmol/L increase: OR = 0.71, 95% CI = 0.59-0.85, $P = 1.71\text{x}10^{-4}$) increased endometrial cancer risk. In the mediation analysis, we found evidence for a mediating role of FI (30% mediated, 95% CI = 10-50%, P = 3.11x10-3), bioavailable testosterone (12% mediated, 95% CI = 7-16%, P = 1.92x10-6), and SHBG (7% mediated, 95% CI = 2-12%, P = 2.72x10-3). Our comprehensive analysis provides insight into potential causal mechanisms linking BMI with endometrial cancer risk and suggests pharmacological targeting of insulinemic and hormonal traits as a promising strategy for endometrial cancer prevention. This research was completed in the MRC Integrative Epidemiology Unit at the University of Bristol (MC_UU_00011/4) and supported by Cancer Research UK (C18281/A29019).

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P19.017.A Using genetics to understand the biological and non biological factors that influence susceptibility to EBV infection

Marisa D. Muckian[1], *Helen R. Stagg*[1], *Graham S. Taylor*[2], *James F. Wilson*[1,3], *Nicola Pirastu*[1]

[1]*University of Edinburgh, Usher Institute, Edinburgh, United Kingdom,* [2]*University of Birmingham, Institute of Immunology and Immunotherapy, Birmingham, United Kingdom,* [3]*University of Edinburgh, Institute of Genetics and Cancer, Edinburgh, United Kingdom.*

Epstein-Barr Virus (EBV) infects >90% of the population. EBV infection is life-long and has been linked to autoimmune conditions as well as cancer. Risk factors for EBV infection are not well understood and the current literature is conflicting.

In order to establish the true EBV risk factors we conducted a GWAS on serostatus along with the measured antibody levels for infectious diseases including EBV in a sample of 9,724 participants of UKBiobank. The results of the GWAS were then used to perform mendelian randomization (MR) to assess the robustness of previously identified EBV risk factors.

Significant associations were found between the HLA locus and all tested EBV antibody levels, confirming previous studies. Of the four loci (all novel) associated with EBV susceptibility, none were located in the HLA region suggesting that being infected with EBV and the immune response to the virus are independent phenomena. MR analysis confirmed several previously hypothesised risk factors such as socioeconomic status, however we found little evidence for many of the others.

We have identified several novel loci associated with EBV that will better our understanding of the biology behind infection and give us potential insight into why some people remain seronegative. Our results show that applying MR to infectious disease studies can help verify the risk factors that are truly associated with serostatus, especially when traditional epidemiological studies provide opposing results.

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P19.018.B Rare variants in the FZD4 gene in patients with familial exudative vitreoretinopathy

Sofya Garifullina[1], *Andrey Marakhonov*[1], *Vitaly Kadyshev*[1], *Tatyana Vasilyeva*[1], *Elizaveta Geraskina*[2], *Sergey Kutsev*[1], *Rena Zinchenko*[1]

[1]*Research Center for Medical Genetics, Moscow, Russian Federation,* [2]*Helmholtz National Medical Research Center of Eye Diseases, Moscow, Russian Federation.*

Familial exudative vitreoretinopathy (FEVR) is a hereditary eye disease caused by mutations in 7 genes (*CTNNB1, TSPAN12, EVR3, ZNF408, LRP5, FZD4, NDP*) (OMIM PS133780). The aim of the study was to analyze the pathogenic variants in the frizzled-4 gene (*FZD4*), which is localized on 11q14.A cohort of 34 patients aged up to 18 years with a 2:3 sex ratio (men:women) with FEVR and characteristic clinical picture (disorders of retinal vascularization, fibrotic changes in the vitreous body, and secondary retinal detachment) was screened for the presence of variants in the *FZD4* gene. Two of 34 patients have heterozygous variants - one novel variant NM_012193.4:c.1486del which leads to frameshift resulting in the formation of a premature translation termination site, p.(Trp496Glyfs*17), and one previously described pathogenic variant c.205C>T leading to a missense substitution p.(His69Tyr) causing incorrect folding of the FZD4 protein. This variant is registered in the Human Mutation Database and is associated with FEVR1.In our study of Russian cohort, two pathogenic variants were identified, one of which was novel. The frequency of *FZD4* pathogenic alleles in our cohort is 2%. That indicates that *FZD4* variants could explain small number of FEVR cases among Russian population, in contrast to other European populations with 20% explained by *FZD4* variants cases. The study suggests seeking for the causes in other genes.The study was supported by the RFBR grant № 19-015-00122 and was carried out as part of the State assignment of the Ministry of Science and Higher Education of the Russian Federation.

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P19.019.C Molecular spectrum of *PCSK9*-based FH in France, the French p.(Ser127Arg) founder variant

Yara Azar[1,2,3], *Yara Abou-Khalil*[1,2,3], *Mathilde Di-Filippo*[4,5], *Alain Carrié*[6], *Sophie Béliard*[7], *Catherine Boileau*[1,3,8], *Marianne Abi-Fadel*[1,2], *Jean-Pierre Rabès*[1,9], *Mathilde Varret*[1,3]

[1]*LVTS, Inserm U1148, Bichat Hospital, Paris, France,* [2]*Laboratory of Biochemistry and Molecular Therapies (LBTM), Faculty of Pharmacy, Pôle Technologie- Santé, Saint Joseph University, Beirut, Lebanon,* [3]*Paris University, Paris, France,* [4]*Department of Biochemistry and Molecular Biology, Hospices Civils de Lyon, Louis Pradel Cardiovascular Hospital, Bron, France,* [5]*INSERM U1060, INSA de Lyon, INRA U1235, Univ Lyon-1, Université de Lyon, Villeurbanne, Oullins, France,* [6]*Sorbonne University, UMR_1166 & ICAN; Department of Biochemistry, APHP. Sorbonne University, La Pitié-Salpêtrière Hospital, Paris, France,* [7]*Department of Nutrition, APHM, La Conception Hospital, Faculty of Medicine, Aix-Marseille University, INSERM, INRAE, C2VN, Marseille, France,* [8]*Genetics department, APHP, Bichat Hospital, Paris, France,* [9]*Biochemistry and Molecular Genetics Laboratory, AP-HP., Université Paris-Saclay, Ambroise Paré Hospital, Boulogne Billancourt; UVSQ, UFR Simone Veil - Santé, Montigny-Le-Bretonneux, France.*

Introduction: *PCSK9* is the third gene involved in familial hypercholesterolemia (FH). The first FH-causing *PCSK9* variant, p.(Ser127Arg), is almost exclusively reported in French patients and represents 67% of the *PCSK9* variants in France. This study aims to characterize the molecular spectrum of *PCSK9*-based FH in France to analyze genotype/phenotype correlations and p.(Ser127Arg) founder effect.

Methods: *PCSK9* variants were searched through a diagnostic approach using Targeted next-generation sequencing (NGS) genes panels. Five families and 22 probands carrying p.(Ser127Arg) were selected for genotyping. Haplotypes were constructed with (1) 12 microsatellites spanning 20 Mb around *PCSK9* (GeneMapper® Software was used to determine the alleles length (pb)) and (2) SNPs in exon 1: p.(Leu21dup), exon 4: c.524-68G>C; c.524-90G>C; c.657+82 A>G (Sanger sequencing) and SNPs in exon 3: c.400-201 A>G, exon 9: c.1664G>A (TaqMan® SNP Genotyping Assays).

Results: Through NGS in FH probands, we identified new *PCSK9* rare variants: p.(Arg215Cys), p.(Asp367His), p.(Glu410Lys), p.(Arg495Trp), p.(Gly516Val), p.(Ala676Gly), all are predicted deleterious, but functional studies are needed to establish their real causative effect. The L11 allele p.(Leu21tri) associated with familial combined hyperlipidemia was found in 17 probands with varying phenotypes, but normal triglycerides levels indicate that this *PCSK9* variant can also lead to bona fide FH. For all p.(Ser127Arg) carriers, a common haplotype of 1.4 Mb was detected with the SNPs and the first 3 microsatellites analyzed. Genotyping the rest of microsatellites is ongoing.

Conclusion: These preliminary results show that p.(Ser127Arg) gain-of-function variant in *PCSK9* could be due to the founder effect in France.

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P19.020.D Exome sequencingof 1293patientsin Russia: new knowledge aboutthestructureofinherited diseases in Russia

Oxana Ryzhkova, *Olga Mironovich, Polina Gundorova, Anna Orlova, Tatiana Cherevatova, Alyona Chukhrova, Viktoria Zabnenkova, Olga Schagina, Varvara Kadnikova, Nailya Galeeva, Alexander Poliakov*

Research Centre for Medical Genetic, Moscow, Russian Federation.

Whole and clinical exome sequencing (WES/CES) are the most popular diagnostic methods. This analysis gives us information about causes of pathologies, diseases structure in different populations and frequencies of variants.

Materials and Methods: DNA of 1293 patients were analyzed by WES (711) or custom CES (582), which included 6300 genes.

Results: Pathogenic variants were identified in 198 (15,3%) cases, likely pathogenic in 386 (29,7%) and variants of uncertain clinical sugnificance in 548 (46,0%). Mutation types and frequencies in most of the genes were as expected. Four diseases not diagnosed before using WES/CES in Russia are of particular interest. Five patients homozygous for c.362dup variant in *FKBP14* gene were found. The variant was identified in 21/5495 healthy controls. Frequency of *FKBP14*-related disease is 1:250000 in Russia. Spastic paraplegia 47 was identified in 4 patients with homozygous variant c.1160_1161del[PG1]. This variant was found in patients with epilepsia/mental retardation and wasn't found in 128 patients with spastic paraplegia. Eight patients had different variants in *GNE* gene. Thus, Nonaka myopathy frequency the same the most frequent in Russia *CAPN3*-related myopathy. Neuromyotonia and axonal neuropathy were observed in three patients with homozygous variant c.110G>C[PG2] in the *HINT1* gene. 30 additional cases of homozygous or compound-heterozygous c.110G>C were found in 316 patients with hereditary motor and

sensory neuropathy (HMSN) type 2. Thus, *HINT1*-related phenotypes are the most frequent form of autosomal-recessive HMSN in Russia.

Conclusions: CES/WES data are helpful for investigation of a population and for clarification of the burden and structure of hereditary pathology.

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P19.021.A association of FTO (rs9939609), LIPC 250 G>A and LPL Ser447Ter gene polymorphisms with obesity in children and adolescents

Lyudmila Valerievna Gutnikov[1], *Alaa H. Abd A.*[2], *Tatiana Pavlovna Shkurat*[1], *Galina Vladimirovna Karantysh*[3], *V N. Prokofev*[3]

[1]*Research Institute of Biology, Rostov-on-Don, Russian Federation,* [2]*academy biology and biotechnology, Rostov-on-Don, Russian Federation,* [3]*Southern Federal University, Rostov-on-Don, Russian Federation.*

The importance of metabolic and genetic factors in the development of childhood obesity remains relevant.The aim of this work was to study the association of the polymorphisms *LPL Ser447ter (C-G)*, *-250 LIPC G>A* and *FTO rs9939609* with obesity in children and adolescents from the Rostov region (Russia).

Methods: The case-control study involved 520 children and adolescents aged 3 to 17 years. The main group consisted of 370 obese children and adolescents and 150 non-obese children and adolescents (control). Genomic DNA samples were isolated from whole blood of patients using the standard phenol-chloroform method. Genotyping of polymorphisms *FTO T/A rs9939609*, *LIPC G/A-250*, and *LPL Ser447Ter* was performed using fragments amplified by PCR. The MDR method was used to assess gene-gene relationships.

Results: Using MDR, models of intergenic interactions of *FTO rs9939609*, *LPL Ser447ter*, and *LIPC -250 G>A* polymorphism with obesity were constructed. When analyzing the duhlocus interaction between *FTO re9939609 T> A* and *LPL Ser447Ter C>G*, an antagonistic character is shown; between loci *FTO re9939609 T> A* and *LPL Ser447Ter C> G*, the nature of the interaction is synergistic, and with the LIPC-250 G>A polymorphism, it is antagonistic at loci *FTO re9939609 T>A* and *LPL Ser447Ter C>G*.Findings. Depending on the inclusion of different loci in the development of obesity, the nature of the relationship between the polymorphisms of these genes can change.*This study was funded by the Ministry of Science and Higher Education of the Russian Federation №0852-2020-0028*.

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P19.023.C An exome-wide analysis of natural genetic variation in the Canary Islands population

Ana Díaz-de Usera[1], *Luis A. Rubio-Rodríguez*[1], *Adrián Muñoz-Barrera*[1], *Beatriz Guillen-Guio*[2], *Almudena Corrales*[2,3], *Antonio Íñigo-Campos*[1], *David Jáspez*[1], *Itahisa Marcelino-Rodriguez*[2,4], *Antonio Cabrera de León*[2], *Rafaela González-Montelongo*[1], *Jose M. Lorenzo-Salazar*[1], *Carlos Flores*[1,2,3]

[1]*Genomics Division, Instituto Tecnológico y de Energías Renovables (ITER), Santa Cruz de Tenerife, Spain,* [2]*Research Unit, Hospital Universitario N.S. de Candelaria, Universidad de La Laguna, Santa Cruz de Tenerife, Spain,* [3]*CIBER de Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Spain,* [4]*Instituto de Tecnologías Biomédicas, Universidad de La Laguna, Santa Cruz de Tenerife, Spain.*

Introduction: In order to provide the basis for an unbiased understanding of traits and diseases, many countries are developing their own catalogs of genetic variation. The Canary Islands (Spain) population exhibits the largest genetic proportion of North African ancestry among the Southwestern European populations. Here we provide, for the first time, an exome-wide analysis of their genetic variation.

Materials and Methods: DNA from 629 unrelated control donors were sequenced by commercial whole-exome enrichment capture kits and a HiSeq 4000 (Illumina) using 75 bp paired-end reads. BWA-GATK v3.8 Best Practices were followed for germline variant calling using the GRCh37/hg19 human genome as the reference. ANNOVAR, Ensembl VEP, and InterVar tools were used for functional annotation.

Results: A total of 340,913 variants were identified by whole-exome sequencing in the target regions, of which 70.1% were exonic. A quarter of them (i.e., 59,883) were absent from the 1000 Genomes Project, gnomAD, and TopMed, and 97.4% of those presented an allele frequency below 0.5%. Out of these, 3,276 were classified as pathogenic/likely pathogenic according to InterVar. Nearly 90% of those had high impact predictions or were loss-of-function variants, being frameshift the most common variation.

Conclusions: The high percentage of novel exome-wide genetic variation in Canary Islanders highlights the need for a specific catalog to improve the pathogenicity classification of variants in this population.

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P19.025.A Percentage of explained variance in alcohol consumption by genetic risk score in the UK Biobank

Xiyun Jiang[1], *He Gao*[2], *Paul Elliott*[2,3], *Raha Pazoki*[1,2]

[1]*Brunel University London, London, United Kingdom,* [2]*Imperial College London, London, United Kingdom,* [3]*Health Data Research-UK London substantive site, London, United Kingdom.*

Introduction: Recent genome-wide association studies (GWAS) identified over 100 alcohol consumption-associated genetic variants. We investigated variance of alcohol consumption explained by genetic factors in various population subgroups including sex, age, and in relation to central tendency.

Materials and Methods: We created an alcohol consumption genetic risk score (GRS) using 105 previously published alcohol consumption genetic variants in GWAS. Using data from 295,189 UK Biobank (UKB) participants, we calculated percentage variance in alcohol consumption (g/day) explained by the GRS in subpopulations including participants with alcohol consumption levels within (1) mean ± 1 standard deviation (SD) of alcohol consumption distribution, (2) mean ± 2 SD and (3) within the whole sample. We additionally investigated explained variance in age and sex-specific subgroups.

Results: GRS was associated with alcohol consumption (0.122; 95% CI = 0.117-0.126) within the UKB. GRS explained less variation in alcohol consumption among participants whose alcohol consumption fall in the centre of the population distribution of alcohol consumption and explained more variation in participants with higher alcohol consumption. Table 1. Additionally, alcohol GRS explained more variation in men compared to women. Alcohol GRS also showed tendency to capture more variation in alcohol consumption of younger participants.

Conclusions: Our results show that variation in alcohol consumption that is explained by alcohol GRS differs in population subgroups and the location of participants in population's alcohol consumption distribution. MRC Rutherford fund (MR/R0265051/2).

Table 1. Percentage variance of alcohol consumption explained by genetic risk score.

Subgroup	Sample size (%)	Adjusted R^2 (%)	Adjusted R^2 (proportion of the whole sample)
Whole sample	295,189 (100)	0.92	1
Alcohol consumption mean ± 1 SD	204,384 (69)	0.45	0.49
Alcohol consumption mean ± 2 SD	278,689 (94)	0.89	0.97
Alcohol consumption quintile 1	59,025 (20)	0.004	-
Alcohol consumption quintile 2	59,196 (20)	0.037	-
Alcohol consumption quintile 3	61,121 (20.7)	0.00	-
Alcohol consumption quintile 4	56,428 (19.1)	0.03	-
Alcohol consumption quintile 5	59,419 (20.1)	0.15	-
Men	134,169 (45)	1.29	-
Women	161,020 (55)	1.07	-
Age group 1(38-52)	97,698 (33)	1.05	-
Age group 2 (53-60)	89,294 (30)	0.94	-
Age group 3 (61-72)	108,197 (37)	0.79	-

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P19.026.B Establishing the Hungarian Genomic Data Warehouse for studying the genetics of healthy ageing and longevity

Vera Várhegyi[1], *Viktor Molnár*[1], *András Gézsi*[1,2], *Péter Sárközy*[2], *Péter Antal*[2], *Mária Judit Molnár*[1]

[1]*Semmelweis University, Institute of Genomic Medicine and Rare Disorders, Budapest, Hungary,* [2]*Budapest University of Technology and Economics, Department of Measurement and Information Systems, Budapest, Hungary.*

Introduction: Population health research is increasingly focused on the genetic determinants of healthy ageing. Until now there was no public resource of whole genome sequences and phenotype data from healthy elderly individuals in Hungary.

Materials and Methods: The Institute of Genomic Medicine and Rare Disorders of the Semmelweis University set up a data collection, the Hungarian Genomic Data Warehouse, by cataloging and analyzing complete genome sequences and related phenotype data of 100 healthy volunteers. The structure of the data warehouse allows interoperability with the most important international research projects on ageing.

Results: 49% of the participants were between 70-80 years old, 36% between 81-90, 14% over 90 years old. The gender ratio was 44-56% between men and women. The proportion of people with higher education is high (46%), 61% of participants played sports for a long time, and 70% never smoked. The parents of the participants also lived a high age, with an average age at death of 74.3 years for fathers and 80.47 years for mothers.

Conclusions: The Hungarian Genomic Data Warehouse provides insight into molecular diagnostics, from individual common polymorphisms, through population-specific variants, to private, individual-specific mutations. This repository is the first public full genome map in Hungary. The data will be available to the public either providing aggregated genetic statistic data or sharing the anonymazied individual genomic and phenotypic data within scientific collaboration using privacy-preserving data analysis methods.The implementation of the project was supported by the National Bionics Program ED_17-1-2017-0009.

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P19.027.C Exploring the causal connection between sleep traits and pain diagnoses

Martin Broberg, *Juha Karjalainen, FinnGen, Hanna M. Ollila*

University of Helsinki, Helsinki, Finland.

Previous studies have found significant links between insomnia and pain symptoms including both general pain and specific diseases such as migraine. As genome-wide association studies (GWAS) have identified disease specific associations, so too has the possibility of bioinformatically exploring the comorbidity and causality between risk-factors and diseases and the influence of genetics. Here, we aimed to elucidate the causality between sleep and pain, and performed both two-sample and one-sample mendelian randomization analyses using GWAS summary statistics. We tested insomnia as a risk factor based on a self-reported cohort (N = 1 331 010) against cohorts associated with different types of pain; general pain (N = 218 379, inverse-variance weighting (IVW) odds ratio (OR) [95% confidence interval (CI)] = 1.47 [1.38-1.58], $P = 4.12\text{x}10^{-28}$), multi-site chronic pain (MCP, N = 387 649, IVW OR [95% CI] = 1.36 [1.32-1.41], $P = 2.04\text{x}10^{-81}$), neuropathic pain (mono+polyneuropathies, N = 269 141, IVW OR [95% CI] = 1.14 [1.09-1.18] $P = 7.80\text{x}10^{-10}$) and trigeminal neuralgia (N = 246 228, IVW OR [95% CI] = 1.18 [1.06-1.31], $P = 0.003$). Conversely, general pain and MCP exhibited a significant increased risk for insomnia (IVW OR [95% CI] = 1.04 [1.01-1.07], $P < 0.05$ and IVW OR 1.32 [1.26-1.38], $P = 1.58\text{x}10^{-36}$, respectively). Results were consistent in sensitivity analyses. Our findings support a bidirectional causal relationship between insomnia and pain. These data support further clinical investigation into the utility of insomnia treatment as a strategy for pain management and vice versa.

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P19.028.D Hereditary haemochromatosis mutations, brain iron imaging and dementia risk in the UK Biobank cohort

Janice L. Atkins[1], *Luke C. Pilling*[1], *Christine J. Heales*[1], *Sharon Savage*[1,2], *Chia-Ling Kuo*[3], *George A. Kuchel*[3], *David C. Steffens*[3], *David Melzer*[1,3]

[1]*University of Exeter, Exeter, United Kingdom,* [2]*University of Newcastle, New South Wales, Australia,* [3]*University of Connecticut, Farmington, CT, USA.*

Background: Brain iron deposition occurs in dementia. In European ancestry populations, the *HFE* p.C282Y variant can cause iron overload and haemochromatosis, mostly in homozygous males. The aim was to estimate p.C282Y associations with brain magnetic resonance imaging (MRI) features plus incident dementia diagnoses during follow-up in a large community cohort.

Methods: UK Biobank European ancestry descent participants (n = 335,909, 40-70 years) were followed up from baseline (2006-2010) via hospitalization records (mean 10.5 years). Participants included 2,890 *p.C282Y* homozygotes. MRI was available in a subset of 28,860 participants (206 *p.C282Y* homozygotes), including T2* measures (lower values indicating more iron).

Results: Male p.C282Y homozygotes had lower T2* measures in areas including the putamen, thalamus, and hippocampus, compared to no HFE mutations. Incident dementia was more common in p.C282Y homozygous men (Hazard Ratio HR = 1·83; 95% CI 1·23 to 2·72, p = 0.003), as was delirium (HR = 1·82, 95% CI 1·21 to 2·72, p = 0·004) compared to those without the mutations. There were no associations in homozygote women or in heterozygotes.

Conclusion: Men with the *HFE* p.C282Y homozygous mutation developed substantially more marked brain iron deposition in dementia relevant brain areas and were more likely to be diagnosed with dementia during follow-up in hospitalization data. Studies are needed of whether early ascertainment of haemochromatosis and iron reduction in *HFE* p.C282Y homozygotes may prevent or limit associated dementia related brain pathologies. **Grant details** - UK Medical Research Council award MR/S009892/1 (Principal investigator David Melzer).

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P19.029.A Wide spectrum of *F9* variants in hemophilia B families from the Portuguese population

Isabel Moreira[1], *Maria J. Diniz*[2], *Alice Tavares*[3], *Sara Morais*[4], *Bruno Freitas*[5], *Fernando Araújo*[6], *Teresa Gago*[7], *Ema M. Antunes*[2], *Cristina Catarino*[3], *Manuel Campos*[4], *Teresa Almeida*[8], *Sara B. Santos*[8], *Raquel Maia*[8], *Paula Kjollerstrom*[8], *João Lavinha*[1], *João Gonçalves*[1], *Dezso David*[1]

[1]*Departamento de Genética Humana - Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa, Portugal,* [2]*Serviço de Imuno-Hemoterapia, Hospital de S. José, Centro Hospitalar de Lisboa Central EPE, Lisboa, Portugal,* [3]*Serviço de Imuno-Hemoterapia, Hospital de Santa Maria, Centro Hospitalar de Lisboa Norte EPE, Lisboa, Portugal,* [4]*Serviço de Hematologia Clínica, Hospital de Santo António, Centro Hospitalar do Porto, Porto, Portugal,* [5]*Serviço de Sangue e Medicina Transfusional, Hospital Central do Funchal, Funchal, Portugal,* [6]*Serviço de Imuno-Hemoterapia, Centro Hospitalar de São João EPE, Porto, Portugal,* [7]*Serviço de Patologia Clínica, Hospital de São Francisco Xavier, Centro Hospitalar de Lisboa Norte EPE, Lisboa, Portugal,* [8]*Serviço de Hematologia Pediátrica, Hospital D. Estefânia, Centro Hospitalar de Lisboa Central EPE, Lisboa, Portugal.*

Introduction: Hemophilia B is an X-linked bleeding disorder caused by molecular defects in the Factor IX gene (*F9*), leading to either deficiency or functional abnormality of Factor IX. Actual data indicate a high heterogeneity of variants in *F9*. Over 1000 different variants have been reported, including pathogenic single nucleotide variants (SNPs), indels and complex variants.

Materials and Methods: 86 index patients and 313 relatives were studied. *F9* variant analysis was performed from total genomic DNA by PCR followed either by SSCP and DNA sequencing or direct DNA sequencing. When no variant was detected by sequencing, *F9* analysis by MLPA was performed. Segregation studies were performed in each family.

Results: Overall, 52 different *F9* variants have been identified, including 49 SNPs or small indels, a gross duplication (exons 2-6) and two deletions of the entire gene. Ten of the variants had been firstly reported by us and three are novel: c.391+5G>T; c.432T>G, p.(Phe144Leu) and c.749C>A, p.(Ala250Glu). This approach allowed establishing the carrier state of over 300 women and 12 prenatal diagnoses were performed.

Conclusions: The spectrum of *F9* variants identified in the Portuguese population significantly overlaps that observed in other populations. Identification of *F9* gene variants in patients allows genotype-phenotype correlations and carrier detection, as well as prenatal diagnosis. Sanger sequencing of the coding region and adjacent intronic sequences of *F9* still remains a valid and effective tool for the molecular study of hemophila B, providing information for appropriate genetic counseling and new insights regarding the molecular basis of the pathology.

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P19.030.B Biochemical, epigenetic and genetic components of high altitude adaptation in Tibetans

Nipa Basak[1,2], *Juan Castillo-Fernandez*[3], *Tsering Norboo*[4], *Lomous Kumar*[1], *Mohammed S. Mustak*[5], *Jordana T. Bell*[3], *Kumarasamy Thangaraj*[1,2,6]

[1]*CSIR-Centre for Cellular and Molecular Biology, Hyderabad, India,* [2]*Academy of Scientific and Innovative Research, Ghaziabad, India,* [3]*Department of Twin Research & Genetic Epidemiology, King's College London, London, United Kingdom,* [4]*Ladakh Institute of Prevention, Leh, India,* [5]*Department of Applied Zoology, Mangalore University, Mangalore, India,* [6]*Centre for DNA Fingerprinting and Diagnostics, Hyderabad, India.*

The Tibetan population is well-known for high altitude adaptation. We studied two groups of Tibetans, one from high altitude (4500 meters above sea level (masl)); another migrated to low altitude (~880 masl) about ~60 years ago. After migration, the second group had been subjected to 'relative hyperoxia' because of environmental shift. We studied various hematological and epigenetic signatures of these two groups. Additionally, we searched for loci that are under natural selection. Hematological parameters of 89 low and 79 high altitude Tibetans were evaluated by automated hematology analyzer and manual methods. Serum erythropoietin was measured by ELISA. GraphPad Prism was used for statistical analysis. We carried out whole genome bisulfite sequencing (WGBS) of 10 Tibetans (5 from each altitude) and

analyzed data in R. XP-EHH and iHS tests were performed after genotyping of 36 Tibetans (18 from each altitude) to detect natural selection. We observed significant differences ($P < 0.05$) between these two groups in various hematological parameters, such as RBC, HCT, Hb, MCV, MCH, and MCHC, however, serum erythropoietin level was not significantly different. WGBS revealed 71 significantly differentially methylated regions between high and low altitude Tibetans ($FDR < 0.05$) with methylation level differences > 15%. Interestingly, several differentially methylated regions map to GWAS genes associated with various hematological parameters (*DUSP22*, *CIZ1*, *TMPRSS6*, *PF4V1*, *LRCOL1* etc). Selection tests revealed genetic variants under natural selection including in *TMEM247*, *EPAS1*, *ATP6V1E2* etc. To summarize, our study reveals biochemical, epigenetic and genetic components of high altitude adaptation in Tibetans.

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P19.031.C Interactions between STAT3 IL10 and IL12B genes polymorphism with viral load among women with human papillomavirus

Abbas Hadi Hammadi Albosale

southern Federal University, Rostov-on-don, Russian Federation.

Introduction: HPV infection leads to imbalance in pro-and anti-inflammatory cytokines which promotes for long-term persistence of the virus in the infected cells.

Materials and methods: In our work, we assessed the association of the polymorphic variants STAT3 G>C (rs2293152), IL-10 -1082G>A (rs1800896) and IL-12B +1188A>C (rs3212227) genes with high-risk HPV infection among of women 30 years and older (104 women with HPV load more than 3 lg and 110 women without HPV). Genotyping for polymorphic variants IL-10 -1082G>A, IL-12B +1188A>C were conducted by allele-specific PCR and restriction fragment length polymorphism (RFLP) for the STAT3. Intergenic interactions analysis was carried out by multifactor dimensionality reduction (MDR).

Results: The study of individual SNP STAT3 G>C, IL-10 -1082G>A and IL-12B +1188A>C did not reveal significant differences in the frequencies of genotypes and alleles between two groups of women. At the same time, the MDR analysis revealed significance of intergenic interactions for allelic variants ($OR = 3.19$, 95% $CI = 1.82\text{-}5.58$; $p = 0.0001$). A statistically significant difference revealed among two groups women for genotype frequencies of STAT3 GG / IL12B 1188CC / IL-10 -1082GA. This genotype considered as a protective factor ($OR = 0.19$, 95% $CI = 0.05\text{-}0.67$; $p = 0.01$) at HPV infection. Genotype STAT3 GC / IL12B +1188CC / IL-10 -1082GA potentially increase the risk of high HPV load ($OR = 3.54$, 95% $CI = 1.24\text{-}10.07$; $p = 0.02$).

Conclusions: significant associations between the three SNPs with a high HPV load indicate that these polymorphisms are potential candidates for predicting risk factors or protective factors for women infected with HPV.

A.H.H. Albosale: None.

P19.032.D Wright and Malékot assessments of Inbreeding in the populations of North Ossetia with subdivided structure

Galina I. Elchinova*, Tatyana A. Vasilyeva, Rena A. Zinchenko*

Research Centre for Medical Genetics, Moscow, Russian Federation.

Inbreeding is the most important population-genetic characteristic. That influences on the load and spectrum of hereditary pathology and the nature of genetic and demographic processes in populations. The multidimensional real-life genetic space is difficult to observe. Its various projections. for example. on the space of surnames or of migrations. allow us to obtain numerical characteristics comparing populations.In the study we used 533313 surnames from the voter lists and a total sample of marriage records for 1990-2000 throughout the Republic of North Ossetia. the North Caucasus. Russia. 13935 of them were included in the analysis. Random inbreeding was evaluated by the Wright method based on the frequency distribution of surnames for populations of the "district" rank. The estimation of local inbreeding was calculated using the Malékot's distance isolation model based on the lengths of mating migrations also for populations of the "district" rank. Table. Random and Local inbreeding

Population	Random inbreeding	Local inbreeding
Sity Vladikavkaz	0.00029	0.000024
Pravoberezhniy raion	0.00058	0.000152
Ardonskiy raion	0.00069	0.000327
Digorskiy raion	0.00088	0.000394
Irafskiy raion	0.00120	0.000587
Prigorodniy raion	0.00101	0.000084
Alagirskiy raion	0.00076	0.000192
Kirovskiy raion	0.00095	0.000333
Mozdokskiy raion	0.00018	0.000095

Random inbreeding ranged from 0.00018 in Mozdokskiy to 0.00120 in Irafskiy raion with average 0.00073. Local inbreeding varied from 0.000024 to 0.000587 with average 0.000242. The coefficient of linear correlation was 0.72 and rank correlation was 0.58. Such a high and significant correlation coefficient between the two estimates obtained in different ways indicates at the agreement of the conducted studies. The results were obtained within the RSF grant № 17-15-01051.

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P19.033.A Identification of new genetics variants in interleukin 6 in admixed populations from south-western Colombia

Cristian Fong[1]*, Laura Cifuentes*[2]

[1]*Universidad Cooperativa de Colombia, Santa Marta, Colombia,* [2]*Universidad Cooperativa de Colombia, Pasto, Colombia.*

Introduction: Interleukin-6 (IL-6) is important gen in the immune response. Variability within this gene has not been assessed in the Colombian population, or if there are unique variants in the population.

Materials and method: Blood samples (4ml) were taken from 150 individuals from three localities in south-western Colombia (Pasto, Policarpa, Magüí Payán) were analyzed. DNA extraction was performed followed by sequencing of all exons and 700 bp of the promoter region of the IL-6 gene. The Blast tool was used to align the resulting sequences with those reported in the GenBank. The allele and genotypic frequency of each variant were used to establish its distribution among the three locations.

Results: twenty variants were identified, of which seven have not been reported in the databases. These new polymorphisms had a frequency between 1-2% in the total population. Five variants including rs1800796 had Fis negative, indicating an excess of heterozygotes. Magüí Payán differed from Pasto and Policarpa (Fst = 0.015, 0.014 respectively), while for Pasto and Policarpa there was no major difference.

Conclusions: This study reports seven new polymorphisms for the IL-6 gene and its promoter region. These polymorphisms may be recent due to the low frequency in the population. Some polymorphisms have a tendency of increasing heterozygosity which may be due to genetic drift or natural selection. Similarly, genetic differences between an Afro-descendant population (Magüí Payán) and mestizo populations (Pasto and Policarpa) may be the result of historical processes or natural selection. This research was funded by Colciencias contract FP44842-122-2016.

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P19.034.B A rare missense variant of the hemojuvelin gene as a founder effect and cause of juvenile hemochromatosis in a First Nation population in Alberta, Canada

Sherryl A. M. Taylor[1,2], *Jeffrey M. Patterson*[3,4], *Malcolm Wells*[5,4], *Juan Gonzalez-Abraldes*[5,4], *Matthew Smith*[5,4], *Farshad Niri*[2], *Lisa Prat*[2], *Alison Eaton*[1,4]

[1]*Department of Medical Genetics, University of Alberta, Edmonton, AB, Canada,* [2]*Genetics and Genomics, Alberta Precision Laboratories, Edmonton, AB, Canada,* [3]*Department of Medicine, Division of Haematology, University of Alberta, Edmonton, AB, Canada,* [4]*University of Alberta Hospital, Alberta Health Services, Edmonton, AB, Canada,* [5]*Department of Medicine, Division of Gastroenterology, University of Alberta, Edmonton, AB, Canada.*

Juvenile hemochromatosis is a rare recessive iron overload disorder (incidence less than 1 in 1 million) with most caused by pathogenic variants of the hemojuvelin (HJV) gene. We have ascertained three individuals, with clinically established iron overload, who are homozygous for a rare missense variant of the HJV gene (gnomAD frequency 1/100,000). This variant, HJV c.442T>A, results in the substitution of a conserved cysteine at position 148 with a serine (p.Cys148Ser, NM_213653.3). When assessed using ACMG criteria it was determined to be Likely Pathogenic. The literature suggests this residue participates in intrachain disulphide bonds necessary for correct protein folding. The affected individuals are Cree from First Nation settlements in northern Alberta (population approx. 1500). These individuals were ascertained as separate kindreds without evidence of consanguinity, suggesting HJV c.442T>A is a founder variant. Homozygotes develop early onset iron overload. As teenagers and young adults they exhibit elevated ferritin levels, as high as 1600 ug/L. Ferritin levels can be normalized by routine phlebotomy. Compliance with treatment is complicated by the remote location. One proband developed cirrhosis and hemachromatosis as a teenager and now as an adult has progressed to hepatic carcinoma. Heterozygous individuals tested to date do not show evidence of increased iron stores. Cascade genetic testing and assessment has been undertaken to identify other affected individuals. These findings provide an opportunity to assess the clinical impact of a rare variant of the HJV gene and to provide assessment and counselling to affected individuals in this Canadian First Nation population.

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P19.035.C The association between lactotransferrin genetic variants and dental caries in Libyan students

Fakhri F. Aljedaemi, *Inas M. Alhudiri, Ghada M. Salem, Hamza Ben Zeglam, Nabil S. Enattah*

Biotechnology Research Centre, Tripoli, Libyan Arab Jamahiriya.

Background: Dental caries is a complex, multifactorial disease and one of the most common diseases worldwide, resulting from the interaction of biofilm, cariogenic diet and host response factors. Lactotransferrin (LTF) is a main salivary glycoprotein, which modulates the host immune-inflammatory and antibacterial response. Recent evidence suggests a role of LTF in caries. The aim of this study was to investigate the association between LTF gene single nucleotide polymorphisms in amino acid positions 29 and 47 and dental caries.

Material and methods: Forty-two 12-years-old Libyan students were selected; 20 with dental caries (DMFT ≥ 1) and 22 without caries (DMFT = 0). DNA samples were analyzed by Sanger sequencing in the region spanning rs# 1126477 and rs#1126478 variants.

Results: The variant rs#1126477 at codon position 29 showed significant association with dental caries (p = 0.04). There was also a positive correlation with milk intake and diabetes. The G Allele frequency in patients with caries was 70.5% compared with 44.7% in caries-free students; while A allele frequency was 21% caries-free and 13% in students with dental caries. No significant association between LTF rs#1126478 variant, and risk of dental caries was observed.

Conclusions: LTF rs#1126477 variant may influence the risk of caries in. In addition, the positive relation with diabetes and milk drinking might contribute to the understanding of the genetic susceptibility of dental caries in humans.

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P19.036.D Investigation of a nonsense mutation located in the complex KIV-2 copy number variation region of apolipoprotein(a) in 10,910 individuals

Silvia Di Maio[1], *Rebecca Grüneis*[1], *Gertraud Streiter*[1], *Claudia Lamina*[1], *Manuel Maglione*[1], *Sebastian Schoenherr*[1], *Dietmar Öfner*[1], *Barbara Thorand*[2], *Annette Peters*[2,3], *Kai-Uwe Eckardt*[4,5], *Anna Köttgen*[6], *Florian Kronenberg*[1], *Stefan Coassin*[1]

[1]*Medical University of Innsbruck, Innsbruck, Austria,* [2]*German Research Center for Environmental Health, Neuherberg, Germany,* [3]*German Centre for Cardiovascular Research, Partner Site Munich Hearth Alliance, München, Germany,* [4]*Friedrich-Alexander Universität Erlangen-Nürnberg, Erlangen, Germany,* [5]*Universitätsmedizin Berlin, Berlin, Germany,* [6]*University of Freiburg, Freiburg, Germany.*

Introduction: Lipoprotein(a) [Lp(a)] is highly atherogenic and mainly genetically determined by the *LPA* gene. Up to 70% of LPA is encoded in a hypervariable copy number variation (CNV) named "kringle IV type 2" (KIV-2) CNV. Genotyping variants within the KIV-2 region requires highly sensitive and costly technologies. Therefore, large epidemiological studies on the previously reported *LPA* KIV-2 p.Arg21Ter mutation are missing.

Material and Methods: We typed p.Arg21Ter in three German populations (GCKD, KORA-F3, KORA-F4, n = 10,910) using multiplex allele-specific TaqMan PCR. Allelic location was determined after allele separation by pulsed-field gel electrophoresis (PFGE).

We identified a proxy SNP in GWAS data, confirmed linkage disequilibrium (LD) experimentally (PFGE) and determined p. Arg21Ter frequencies in the 1000 Genomes project (1000G).

Results: p.Arg21Ter (carrier frequency: 1.6-2.1%) occurs on medium sized *LPA* alleles and associated with lower Lp(a) (β = -11.7 mg/dL [-15.5;-7.8], p = 3.39e-32) in a fixed-effect linear regression meta-analysis. In the 1000G data, carrier frequency was ≈0.024 and ≈0.019 in Europeans and South-Asians, with no carriers in Africans and East-Asians. Strikingly, the best proxy SNP was another *LPA* loss-of-function mutation (rs41272114, D'=0.958, R^2=0.281, MAF = 2.6%). D' was close to 1 also in all 1000 G populations but frequencies diverged drastically.

Conclusions: p.Arg21Ter associates with lower Lp(a) and is in LD with the more frequent *LPA* loss-of-function mutation rs41272114 in all investigated populations. Despite its clear molecular function, the p.Arg21Ter genotype does not provide additional phenotypic information beyond rs41272114 genotype. This underscores the importance of linkage disequilibrium patterns even for seemingly clear-cut loss-of-function mutations. Austrian Science Fund (FWF) P31

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P19.037.A Discordant phenotype sequencing of *LPA* proposes a cumulative impact of multiple genetic variants in determining extraordinary high lipoprotein(a) concentrations

Rebecca Grüneis[1], *Claudia Lamina*[1], *Peter Zöscher*[1], *Sebastian Schönherr*[1], *Monika Summerer*[1], *Patricia Döttelmayer*[1], *Lukas Forer*[1], *Kai-Uwe U. Eckardt*[2,3], *Anna Köttgen*[4], *Florian Kronenberg*[1], *Stefan Coassin*[1]

[1]*Institute of Genetic Epidemiology, Department of Genetics and Pharmacology, Medical University of Innsbruck, Innsbruck, Austria,* [2]*Department of Nephrology and Hypertension, Friedrich-Alexander Universität Erlangen-Nürnberg and German Chronic Kidney Disease study, Erlangen, Germany,* [3]*Department of Nephrology and Medical Intensive Care, Charité – Universitätsmedizin Berlin, Berlin, Germany,* [4]*Institute of Genetic Epidemiology, Faculty of Medicine and Medical Center, University of Freiburg, Innsbruck, Austria.*

Introduction: The *LPA* gene encodes apolipoprotein(a) and is the main genetic regulator of lipoprotein(a) [Lp(a)] concentrations. It consists of a CNV, resulting in >40 protein isoforms. Short isoforms show a 5-10 times higher median Lp(a) concentration than long isoforms and doubled cardiovascular disease risk. However, every isoform group presents by isoform unexplained Lp(a) ranges.

Methods: To identify variants associated with extraordinary high Lp(a), a selection based only on concentrations is ineffective, given their dependency on isoforms. After excluding known Lp(a) modifying variant carriers, 96 individuals from the German Chronic Kindey Disease study with high Lp(a) in the top 10% of residuals from a multivariable linear model on Lp(a) including age, sex, eGFR and shorter isoform were selected. 96 controls from the middle 20% residuals were matched and *LPA* was sequenced. Three Lp(a)-SNP scores capturing effects of up to 2,462 SNP over a 2 MB region around *LPA* were computed.

Results: Multiple frequent variants (MAF: 0.167 [IQR: 0.157, 0.167]), that are mostly absent in imputed genome datasets, show stronger effects on Lp(a) in cases (β = 31.91 mg/dL [IQR: 28.34, 47.79]). These effects were, however, due to LD with isoforms, while no variant segregated with case-control status. In contrast, cases show higher SNP scores compared to controls (p = 0.0045).

Conclusion: Results indicate increased Lp(a) levels as a cumulative effect of multiple variants. The discrepancy between sequencing data and SNP score may suggest still unknown regulatory regions located more far apart from *LPA*.

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P19.038.B *MRE11A* locus rs533984 - A marker of selective survival up to the age 85+ in Croatian population

eljka Celinćak, *Maja etinc, Luka Bočkor, Anita Stojanović Marković, Matea Zajc Petranović, Marijana Peričić Salihović, Tatjana karić-Jurić*

Institute for Anthropological Research, Zagreb, Croatia.

Introduction: Human longevity is a multifactorial characteristic, influenced by both genetic and environmental factors. This study aimed to explore whether any difference in longevity genes' makeup could be found in two extreme age cohorts originating from the same population.

Materials and Methods: 42 SNPs, selected due to their strong and replicated relation to human longevity and their involvement in different metabolic pathways, were genotyped in a Croatian study sample consisting of 411 individuals. Allele and genotype frequencies were compared between 314 individuals aged 85+ and 97 individuals aged 20-35 years.

Results: The allele (p = 0.002) and genotype (p = 0.006) frequencies differed only in the rs533984 of the *MRE11A* gene belonging to the DNA repair pathway, with the longevity allele G being more frequent in the old cohort. A marginal difference is also found for the *ApoE* rs7412 allele frequency (p = 0.049), with the longevity allele T (determining ε2 isoform) being more frequent in the old cohort. The G allele of rs533984 has been previously confirmed as favourable for surviving to very old age in Danish females. However, this is the first time to our knowledge that the allele and genotype frequencies of rs533984 have been found to differ between old and young cohorts.

Conclusions: The differences in allele and genotype distribution between two extreme age groups of the Croatian population open a possibility that the G allele of the *MRE11A* gene rs533984 locus might contribute to positive age-related selective survival. **Funding:** Croatian Science Foundation (IP-01-2018-2497).

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P19.039.C Two frequent variants hidden in the *LPA* KIV-2 copy number variation lower lipoprotein(a) concentration and protect against coronary artery disease

Johanna Franziska Schachtl-Riess[1], *Azin Kheirkhah*[1], *Rebecca Grüneis*[1], *Silvia Di Maio*[1], *Sebastian Schoenherr*[1], *Gertraud Streiter*[1], *Jamie Lee Losso*[1], *Bernhard Paulweber*[2], *Kai Uwe Eckardt*[3,4], *Anna Köttgen*[5], *Claudia Lamina*[1], *Florian Kronenberg*[1], *Stefan Coassin*[1]

[1]Medical University of Innsbruck, Innsbruck, Austria, [2]Paracelsus Private Medical University, Salzburg, Austria, [3]Friedrich-Alexander Universität Erlangen-Nürnberg, Erlangen, Germany, [4]Charité - Universitätsmedizin Berlin, Berlin, Germany, [5]University of Freiburg, Freiburg, Germany.

Introduction: Lipoprotein(a) [Lp(a)] is a major genetic risk factor for coronary artery disease (CAD). The *LPA* gene (encoding apolipoprotein(a)) explains >90% of Lp(a) variance. 30-70% is explained by a large coding copy number variation ("KIV-2 repeat") encompassing up to 70% of *LPA*. It generates >40 isoforms. Little is known about the role of variants in the KIV-2.

Methods: We typed a novel putative splicing mutation ("KIV-2 4733G>A") in the German Chronic Kidney Disease study (n = 4,673) by allele-specific real-time PCR and created minigenes. Proxy SNPs were used to analyze impact on CAD in UK Biobank (n = 440,234). Frequencies in 1000Genomes were determined. The effect of compound heterozygosity with another frequent KIV-2 splicing mutation (4925G>A) was assessed.

Results: 4733G>A (38.3% carriers) is the second strongest genetic factor besides the CNV already explaining 10% of Lp(a) variance. It reduces isoform expression without preventing protein production and lowers Lp(a) by 14.0 mg/dL ([95%CI:15.3-12.6], p = 4.82e-184). Minigenes propose splicing modification. Frequencies differ notably between ethnic groups. Compound heterozygosity with 4925G>A (4.6%) lowers Lp(a) by 41.6 mg/dL. By blunting both alleles it narrows the inter-quartile range from 41.1 to 4.6 mg/dL. In UK Biobank, 4733G>A and compound heterozygosity with 4925G>A are associated with a lower hazard ratio for CAD (9% [95%CI:7-11%] and 12% [95%CI:716%] (both $p < 0.001$)).

Conclusions: Functional variants hidden in the *LPA* KIV-2 CNV have a profound impact on Lp(a) concentrations and CAD risk. A moderate but lifelong genetic Lp(a) reduction translates to a noticeable CAD risk reduction.

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P19.041.A Identification of genetic variants associated with blood miRNA expression levels in children

Geòrgia Escaramís[1,2], Marta Vives-Usano[3,4,5], Carles Hernandez-Ferrer[3,4,5], Léa Maitre[3,5,2], Sandra Andrusaityte[6], Ángel Carracedo[7,8], Maribel Casas[3,5,2], Leda Chatzi[9], Regina Grazuleviciene[6], Kristine B. Gutzkow[10], Solène Cadiou[11], Johanna Lepeule[11], Dan Mason[12], Gillian Santorelli[12], Rémy Slama[11], Marina Vafeiadi[13], Xavier Estivill[4,5,2], Juan R González[3,5,2], Eulàlia Martí[1,2], Martine Vrihjeid[3,5,2], ***Mariona Bustamante***[3,5,2]

[1]Departament de Biomedicina, Institut de Neurociències, Universitat de Barcelona, Barcelona, Spain, [2]CIBER Epidemiología y Salud Pública (CIBERESP), Madrid, Spain, [3]ISGlobal, Barcelona, Spain, [4]Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Barcelona, Spain, [5]Universitat Pompeu Fabra (UPF), Barcelona, Spain, [6]Department of Environmental Sciences, Vytautas Magnus University, Kaunas, Lithuania, [7]Grupo de Medicina Xenómica, Fundación Pública Galega de Medicina Xenómica, Instituto de Investigación Sanitaria de Santiago de Compostela (IDIS), SERGAS, Santiago de Compostela, Spain, [8]Centro de Investigación en Red de Enfermedades Raras (CIBERER) y Centro Nacional de Genotipado (CEGEN-PRB3-ISCIII), Universidade de Santiago de Compostela, Santiago de Compostela, Spain, [9]Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA, [10]Department of Environmental Health, Norwegian Institute of Public Health, Oslo, Norway, [11]University Grenoble Alpes, Inserm, CNRS, Team of Environmental Epidemiology Applied to Reproduction and Respiratory Health, IAB, Grenoble, France, [12]Bradford Institute for Health Research, Bradford Royal Infirmary, Bradford, United Kingdom, [13]Department of Social Medicine, University of Crete, Crete, Greece.*

Genetic and environmental factors influence complex phenotypes in humans; but they are difficult to identify. Molecular endophenotypes such as miRNA expression levels, which are more specific and closer to the genomic effects, might facilitate the identification of genetic determinants. However, cell type heterogeneity within a tissue is an important source of variation of such endophenotypes and it has to be taken into consideration. The objective of this project is the identification of genetic polymorphisms associated with blood miRNA expression levels in cis (miQTLs - quantitative trait loci in a 2 Mb window) in children, and to identify the specific blood cell-types driving the observed associations. The project is built on existing data of 924 European children from the The Human Early-Life Exposome (HELIX) Project. Expression levels of >1000 miRNAs in blood were assessed with the Agilent SurePrint Human miRNA rel21 array, and genotyping was conducted with the Illumina GSA array plus imputation up to 7 million common SNPs with the Haplotype Reference Consortium (HRC) panel. In a preliminary analysis with MatrixQTL we identified 10 cis miQTLs at 5% False Discovery Rate, all localized in the hsa-miR-197-5p locus. Blood cell deconvolution will be conducted using gene expression and DNA methylation data, and cell-interacting miQTLs will be identified using the TensorQTL tool. The dissection at molecular level proposed in this project will help to elucidate genetic mechanisms of complex diseases.FUNDING: ISCIII-ERDF (PI17/01225); EU FP7/2007-206 (no 308333: HELIX)

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P19.043.C Analysis of mitochondrial DNA sequences from the peoples of Daghestan living at different altitudes

Maria V. Golubenko[1], *Alexey A. Zarubin[1], Nadezhda P. Babushkina[1], Natalia V. Tarasenko[1], Ramil R. Salakhov[1], Magomed O. Radzhabov[2,3], Vadim A. Stepanov[1]*

[1]Research Institute of Medical Genetics, Tomsk National Research Medical Center, Tomsk, Russian Federation, [2]Institute of Ecological Medicine, Daghestan State Medical University, Makhachkala, Russian Federation, [3]Institute of Physics, Daghestan Federal Research Center, Makhachkala, Russian Federation.

Introduction: Some mtDNA polymorphisms may be significant for adaptation to hypoxic conditions, such as living at high altitudes. Daghestan Republic is region of Russia with high ethnic diversity, and some populations are located at the heights up to 2500 m. The aim of the study was to compare mtDNA polymorphism in the people living at different heights.

Materials and methods: Mitochondrial DNA was sequenced (Illumina technology) in 170 individuals belonging to different Daghestani ethnicities. The samples were divided into two groups: "highland" (N = 80; 1900 m and more above sea level), and

"lowland" (N = 90; 600-1850 m). The Elson neutrality test was carried out in the mtPhyl program.

Results: In the highlands, haplogroups T2 and H13 were the most frequent (12.5% and 11.25%), and in the lowlands, the most abundant were the haplogroups H15 and H* (14.44% each). The differences were significant ($p < 0.05$) for the haplogroups H13, HV4b, and H*. Haplogroups HV4b, I, U1, R2, C4a, W* were registered only in the highland samples. The Elson neutrality test did not reveal any deviations from neutrality in the "highland"; in the "lowland" sample, the test revealed a deviation from neutrality for the MTND6 ($p = 0.007$) and MTCO2 ($p = 0.02$) genes. For the conservation indices of the missense substitutions, no significant differences were found.

Conclusion: The mtDNA polymorphism differences between the highland and lowland inhabitants suggest that high altitude adaptation might play a role in the substantial genetic differentiation of the Daghestani populations. The study was supported by RFBR grant 19-04-01322-A.

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P19.044.D A billion-year trend of amino acid substitutions in the mitochondrial genome

Alina A. Mikhailova[1,2], *Alina G. Mikhailova*[2,3], *Victor Shamanskiy*[2], *Kristina Ushakova*[2], *Alima Galieva*[2], *Valeria Lobanova*[2], *Valeria Timonina*[2], *Valerian Yurov*[2], *Maria Olyanich*[2], *Dmitry Iliushchenko*[2], *Aleksandr Smirnov*[4], *Ilya Mazunin*[5,6], *Leonard Polishchuk*[7], *Dmitry Knorre*[7], *Konstantin Khrapko*[8], *Konstantin Gunbin*[9,10,11], *Jacques Fellay*[12], *Masashi Tanaka*[13], *Konstantin Popadin*[2,12]

[1]*University of Münster, Münster, Germany,* [2]*Immanuel Kant Baltic Federal University, Kaliningrad, Russian Federation,* [3]*Vavilov Institute of General Genetics, Moscow, Russian Federation,* [4]*Kaliningrad State Technical University, Kaliningrad, Russian Federation,* [5]*Skolkovo Institute of Science and Technology, Skolkovo, Russian Federation,* [6]*Fomin Women's Health Clinic, Moscow, Russian Federation,* [7]*Lomonosov Moscow State University, Moscow, Russian Federation,* [8]*Northeastern University, Boston, MA, USA,* [9]*Institut Gustave Roussy, Villejuif, France,* [10]*Novosibirsk State University, Novosibirsk, Russian Federation,* [11]*Institute of Molecular and Cellular Biology, Novosibirsk, Russian Federation,* [12]*Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland,* [13]*Juntendo University Graduate School of Medicine, Tokyo, Japan.*

It has been shown that the rates of reciprocal amino acid substitutions in prokaryotic and eukaryotic organisms are not balanced leading to the long-term increase (i.e. 'gainers') or decrease (i.e. 'losers') in the frequency of some amino acids. However, the evolutionary driving forces establishing this trend are still unknown. Here, focusing on the strongly asymmetrical mutational spectrum of the mitochondrial genome (an excess of G to A and T to C, light chain notation), we predicted the preferential direction of amino acid substitutions from losers (LeuTT, Phe, Cys, Trp, Gly, and Val) to gainers (Pro, His, Gln, Asn, Lys, and Thr). Analyzing collections of nonsynonymous mtDNA mutations from human cancers (PCAWG), human pathogenic mutations (MitoMap database), human population polymorphisms, and mtDNA polymorphism from hundreds of vertebrate species, we observed that the vast majority of substitutions are indeed in the expected direction: from losers to gainers. Moreover, the observed bias is the most pronounced in datasets where mutagenesis is stronger than selection (cancer and human pathogenic mutations for example). Comparing the amino acid composition of mtDNA genes between orthologs of mitochondrial genes in alpha-proteobacteria, fungi, plants, invertebrates, and five classes of vertebrates, we observed a global billion-year trend: losers become rarer while gainers become more frequent among these taxa. These results are in line with the accumulation of slightly-deleterious variants (i.e. from losers to gainers) in mtDNA from the moment of endosymbiosis emergence till the current days due to genetic drift which becomes stronger from bacteria to vertebrates.

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P19.047.C Association between genes *LPL Ser447Ter* and *FTO rs9939609* polymorphisms with obesity in children and adolescents

Alaa Hashim Abd Ali[1,2], *Olga Vladimirovna Bocharova*[3], *Tatiana Pavlovna Shkurat*[4]

[1]*South Federal University, Academy of Biology and Biotechnology, Rostov-on-Don, Rostov, Russian Federation,* [2]*Al-Furat Al-Awsat Technical University, College of Health and Medical Techniques, Department of Medical Laboratory Techniques, Kufa, Iraq,* [3]*Rostov State Medical University, Rostov-on-Don, Russian Federation,* [4]*South Federal University, Academy of Biology and Biotechnology, Rostov-on-Don, Russia, Russian Federation.*

Introduction: Due to the high prevalence of obesity-related diseases, the importance of researching the relationship between gene polymorphisms and obesity does not lose importance. This study aimed correlation between of the *LPL* and *FTO* genes with obesity in children and adolescents from the Rostov region (Russia).

Methods: In a case-control study, we studied the relationship between the *FTO* and *LPL* genes with obesity in 520 children and adolescents of both sexes aged 3 to 17 years: the main group consisted of 370 obese and control - 150 in children and adolescents. Genotyping of the *FTO* and *LPL* genes were performed using PCR-amplified fragments. These genes in DNA samples were typed by the electrophoretic method using systems from the Litekh research and production company (Russia).

Results: Differences ($P < 0.05$) were revealed between the obesity and control groups in the frequency of the AA genotype ($P = 0.0079$; OR 0.53; 95% CI 0.36-0.79) and allele A ($P = 0.005$; OR 0.67; 95% CI 0.51–0.88) of the *FTO* gene. While the frequencies genotypes of *LPL* gene did not differ in the both groups: the CC genotype was detected in 313 (84.6%) obese and in 125 children and adolescents in the control group (83.3%) ($P = 0.779$; OR 1,10; 95% CI 0.66 - 1.83). Also, We found of the GG genotype was not found in both groups risk in the child and adolescent population of Rostov-on-Don were.

Conclusions: The relationship between the gene *FTO* rs9939609 polymorphism and obesity was revealed.

A.H. Abd Ali: None. **O.V. Bocharova:** None. **T.P. Shkurat:** None.

P19.048.D Genetic variants of *FTO, MAO-A* and *COMT* and personality traits in children with obesity and with normal weight from Yucatán, Mexico

Lizbeth González-Herrera[1,2], *Luis A. Vázquez-Pérez*[1], *Andrés Guzmán-Aguilar*[1], *María José López-González*[3], *Gerardo Pérez-Mendoza*[1], *Gloria Arankowsky-Sandoval*[1], *Monica Hattori-Hara*[4], *Rodrigo Rubi-Castellanos*[1]

[1]Universidad Autónoma de Yucatán, Merida, Mexico, [2]DIMYGEN Laboratorio S.C.P., Mérida, Mexico, [3]DIMYGEN Laboratorio S.C.P., Merida, Mexico, [4]Secretaria de Educación del Gobierno del Estado de Yucatán (SEGEY), Merida, Mexico.

Introduction: Genetic variations that control the availability of dopamine are involved in intake of palatable foods in children and being associated to obesity through loss of control of satiety, impulses and the manifestation of addictive eating behaviors, including personality traits. The *3R-MAOA* low-activity allele was associated with body mass index (BMI). Carriers of the variant Val158Met-*COMT* showed high consumption of foods high in lipids. Children with at least one risk allele for SNP *rs9939609-FTO* showed more frequent episodes of loss of control over food and chose foods rich in energy.

Material and Methods: We genotyped *rs9939609-FTO*, *VNTR-MAOA*, and *rs4680-COMT* in children with and without obesity. Food preferences were determined with Child Eating Behavior Questionnaire (CEBQ). Personality traits: anxiety and impulsiveness with Conner's test, pursuit of high (PH) and low (PL) intensity pleasure with Temperament in Middle Childhood Questionnaire (TMCQ).

Results: Frequency of heterozygous *FTO-rs9939609* ($p = 0.013$) and *COMT-rs4680* ($p = 0.02$), as well as homozygous 3R/3R *MAO-A* ($p = 0.03$) we significantly higher in girls with obesity. For personality traits, mean scale of PL was significantly higher in homozygous AA rs9939609-FTO girls ($p = 0.001$) whereas mean scale of PH was significantly higher in homozygous boys ($p = 0.034$). Mean scale of impulsiveness was also higher for boys ($p = 0.010$) carrying 3R allele of low transcriptional MAO-A activity.

Conclusion: SNPs *rs9939609-FTO*, *VNTR-MAOA*, and *rs4680-COMT* are associated to the risk of obesity only in girls. *rs9939609-FTO* is associated to PL in girls and to PH in boys, whereas 3R *allele-MAOA* is associated to impulsiveness only in boys from Yucatán, Mexico.

L. González-Herrera: None. **L.A. Vázquez-Pérez:** None. **A. Guzmán-Aguilar:** None. **M. López-González:** None. **G. Pérez-Mendoza:** None. **G. Arankowsky-Sandoval:** None. **M. Hattori-Hara:** None. **R. Rubi-Castellanos:** None.

P19.049.A Causality between physical activity, sedentary behavior and obesity: A Mendelian randomization study

Germán D. Carrasquilla[1], *Mario García-Ureña*[1], *Tove Fall*[2], *Thorkild I. A. Sørensen*[1,3], *Tuomas O. Kilpeläinen*[1]

[1]Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, [2]Molecular Epidemiology, Department of Medical Sciences, Science for Life Laboratory, Uppsala University, Upssala, Sweden, [3]Department of Public Health, Section of Epidemiology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark.

Introduction: Observational evidence suggest that physical inactivity leads to weight gain, but some studies indicate reverse causality where weight gain leads to inactivity. As observational studies suffer from confounding and reverse causality, it is challenging to assess the true causal effect direction. We aim to assess the causality between physical activities, sedentary behavior and body mass index (BMI) in adults by bidirectional Mendelian randomization analyses.

Methods: We used results from the largest genome-wide association studies of European ancestry for accelerometer-based physical activity and sedentary time in up to 91,105 individuals, and for BMI in up to 694,649 individuals. We implemented Mendelian randomization analyses using CAUSE method instrumenting full genome-wide association loci. In addition, when using only genome wide significant loci, we obtained estimates using IVW, MR-Egger, weighted median, and weighted mode methods.

Results: We found evidence with causal effects of higher vigorous and moderate physical activity, and less sedentary time on reducing BMI ($P = 2x10\text{-}5$, $P = 0.002$ and $P = 0.02$, respectively). Genetically predicted BMI was linked to more sedentary time ($P = 6x10\text{-}4$), indicating bidirectional causality. We did not find evidence of a causal association between higher BMI and lower levels of vigorous physical activity ($P = 0.11$) or moderate physical activity ($P = 0.25$).

Conclusions: Our results suggest that higher levels of physical activity and less sedentary time are causally associated with lower BMI in adults, supporting the view that lifelong programs for increasing physical activity and reducing sedentary time are beneficial for weight management. **Grants**: Horizon 2020 (No846502), Novo Nordisk Foundation (NNF18CC0034900, NNF17OC0026848), Danish Diabetes Academy (NNF17SA0031406).

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P19.050.B Are highly pleiotropic variants of human traits enriched in genomic regions with strong background selection?

Irene Novo, *Eugenio López-Cortegano, Armando Caballero*

Centro de Investigación Mariña, Universidade de Vigo, Vigo, Spain.

Introduction: Pleiotropic variants, i.e. those that affect more than one trait, have been found to be abundant in the human genome. It has been observed that rare variants tend to be less pleiotropic than common ones, which suggests that highly pleiotropic variants with large effect sizes are subjected to strong purifying selection. However, highly pleiotropic variants are found to have larger effect sizes than less pleiotropic ones, what seems to be contradictory with the purifying selection hypothesis. Thus, we investigated if highly pleiotropic variants are enriched in regions with stronger levels of background selection.

Methods: We evaluated pleiotropy variants affecting 41 human complex traits using data from the NHGRI-EBI GWAS Catalog, and also data from other studies. We analyzed the relationship between the degree of pleiotropy of variants and the intensity of background selection (selection against deleterious mutations) across the human genome.

Results: We found that 23% of the variants analyzed are pleiotropic and that there is a positive correlation between the degree of pleiotropy and the frequency and effect sizes of variants. Our results suggest that the degree of pleiotropy is negatively correlated with the strength of background selection. However, more extensive data from other studies suggest the opposite trend.

Conclusions: Although some of the results found are contradictory, it appears that highly pleiotropic variants are subjected to higher levels of purifying selection than less pleiotropic ones. Funding: AEI (CGL2016-75904-C2-1-P), FPU grant (Ministerio de Universidades, Spain), Xunta de Galicia (ED431C 2020/05) and Fondos Feder.

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P19.051.C Molecular Landscape of different RASopathies in the Cypriot population

Ouranio Anastasiou[1], *Andri Miltiadous*[1], *Petroula Gerasimou*[1], *Jason Chi*[1], *Yiannos Kyprianou*[1], *Andri Mitsidou*[1], *Aimilia Athanasiou*[2], *Irene Savvidou*[2], *Violetta Christophidou Anastasiadou*[2], *Paul Costeas*[1]

[1]*Karaiskakio Foundation, Nicosia, Cyprus,* [2]*Archbihsop Makarios III Children's Hospital, Nicosia, Cyprus.*

The RASopathies are a group of genetic condition caused by germline and/or somatic mutations in genes associated with MAPK pathway. Alterations in these genes result in the development of a group of well-characterized syndromes with overlapping features known as RASopathies. The RASopathies are one of the largest known groups of malformation syndromes and affect approximately 1:1000 individuals worldwide. Here we present the first study in the Cypriot population with respect to different RASopathies. NF1 is excluded from this study since an exclusive study of this gene in the Cypriot population has been previously performed. 43 patients with suspected or confirmed clinical diagnosis of RASopathies, were screened with NGS and MLPA in an attempt to elucidate the underlying genetic etiology of their condition. Of these, 60% had a clinical diagnosis of Noonan syndrome, whereas 12% had a clinical diagnosis of either Costello, NF2, Swannomatosis, Legius or cardio-facio-cutaneous syndromes. Mutations in PTPN11, SOS1 and SOS2 genes were also detected in the 31% of the patients with Noonan syndrome. Mutations in HRAS and NRAS gene were identified in the 7%, mutations in NF2 and SPRED1 in the 7% and mutations in MAF, RIT1, BRAF, SASH1, RASA1, LZTR1, WRN and mTOR genes were detected in the remaining 31% of the patients. This is the first study of the molecular landscape of patients with RASopathies in Cyprus performed with major aim to shed light on the molecular characterization of these patients and the deeper understanding of their precise phenotypic characteristics.

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P19.052.D Longer telomeres are not a positive indicator of extreme longevity (95 years and above) in long-lived individuals

Maja Šetinc*, eljka Celinćak, Luka Bočkor, Nina Smolej Narančić, Tatjana karić-Jurić*

Institute for Anthropological Research, Zagreb, Croatia.

Introduction: Telomere shortening is one of the best researched causes of cellular aging, and a positive relation of longer telomeres with human longevity is found in many studies. This study aimed to explore whether relative telomere length (RTL) is a good biomarker for extreme longevity in long-lived individuals (85+ years). Additionally, the relation of RTL and longevity genes is tested.

Materials and Methods: RTL was measured for 314 Croatian individuals aged 85 years and upwards, and their ages at death were determined 10 years later. 42 SNPs were selected due to their prior association with human longevity and genotyped for this sample.

Results: In this group of elderly individuals a negative correlation of RTL and age at death ($r = -0.114$; $p = 0.043$) is found, and binary logistic regression indicates longer RTL as a negative predictor ($p = 0.024$) for reaching 95 years of age. The multivariate logistic regression analysis showed that 42 selected longevity genes' loci explained 34.4% of RTL variance. It also pointed to *SH2B3* rs3184504 ($p = 0.007$) and *LPA* rs10455872 ($p = 0.027$) being significantly related to RTL. This relation was confirmed by t-test showing significant differences in mean RTL among genotypes: both the TT homozygote of rs3184504 and AA homozygote of rs10455872 were related with shorter RTL.

Conclusions: For long-lived individuals telomere length is not a positive predictor for the age of death, especially for the oldest old category. Further studies are needed to explore the impact of various longevity genes on RTL in elderly individuals. **Funding:** Croatian Science Foundation (IP-01-2018-2497).

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P19.054.B Heterozygote selection against ID alleles may shape the landscape of autosomal-recessive pathogenic variants in European populations

Hila Fridman[1,2,3], *Helger G. Yntema*[4], *Reedik Mägi*[5], *Reidar Andreson*[5], *Andres Metspalu*[5], *Massimo Mezzavila*[6], *Chris Tyler-Smith*[7], *Yali Xue*[7], *Shai Carmi*[2], *Ephrat Levy-Lahad*[1,3], *Christian Gilissen*[4], *Han G. Brunner*[4,8]

[1]*Shaare Zedek Medical Center, Jerusalem, Israel,* [2]*Braun School of Public Health and Community Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel,* [3]*Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel,* [4]*Department of Human Genetics, and Donders Center for Neuroscience, Radboud University Medical Centre, Nijmegen, Netherlands,* [5]*Estonian Genome Centre, Institute of Genomics, University of Tartu, Tartu, Estonia,* [6]*Institute for Maternal and Child Health IRCCS Burlo Garofolo, Trieste, Italy,* [7]*The Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, United Kingdom,* [8]*Department of Clinical Genetics and GROW-School for Oncology and Developmental Biology, and MHeNS School for Neuroscience, Maastricht University and Maastricht University Medical Center, Maastricht, Netherlands.*

Background: In consanguineous populations autosomal-recessive (AR) mutations are a major cause of intellectual disabilities (ID), but in outbred populations new mutations explain the majority of cases. Here we investigated this phenomenon by studying the distribution of alleles and the effect of consanguinity in different groups of disorders.

Methods: We used 6447 exome-sequences of healthy, genetically unrelated Europeans of two distinct ancestries (Dutch and Estonian), and calculated the at-risk couples (ARCs) rates for 1929 AR genes. We compared these rates to expected ARCs rates in first-cousin couples.

Results: We estimate that 0.8-1% of European couples are at-risk of having a child affected with a severe AR genetic disorder. This overall risk is 16.5-fold higher for first-cousins, but the increase varies for different disorders. Notably, the risk is much more increased for skeletal disorders (120x) and ID (40x) in comparison to all other disorders. We find that this significant increase reflects a distinct genetic architecture of pathogenic alleles. In 1000-Genomes data, we found stronger patterns of negative selection on ID and skeletal disorders than on other recessive disorders. Simulations show that even a modest effect on heterozygote fitness could explain this distinct genetic architecture of ID and skeletal pathogenic alleles.

Conclusions: Our results show that ID and skeletal disorders have a unique genetic architecture compared to other disorders. Modeling suggests that this architecture could be explained by a small negative effect on fitness for heterozygous carriers of pathogenic variants in the genes underlying these disorders.

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P19.055.C Genetic markers associated with Alzheimer's disease and schizophrenia demonstrate deviation from selective neutrality in populations of North Eurasia

Anna Bocharova, *Vadim Stepanov*

Research Institute of Medical Genetics, Tomsk National Research Medical Center, Tomsk, Russian Federation.

Introduction: Positive selection during human dispersal was, probably, one of the mechanisms that led to the accumulation of high frequencies of risk alleles for common complex diseases. Along with genetic functional-related effect, population-related differences also may play important role in the genetic variability of common complex diseases (such as Alzheimer's disease and schizophrenia). Due to the lack of information on genetic variability in native populations of North Eurasia for genes associated with psycho-neurological diseases, they are of great scientific interest.

Materials and Methods: Thirty SNPs associated with Alzheimer's disease and schizophrenia were genotyped by MALDI-TOF mass-spectrometry using MassARRAY Analyzer 4 (Agena Bioscience) in sixteen populations of North Eurasia (Russians, Udmurts, Kazakhs, Uzbeks, Kyrgyz, Yakuts, Kets, Northern Altaians, Southern Altaians, Evenks, Buryats, Khants, Tuvinians, Khakass, Chukchi, Nivkhs, Koryaks). Selective neutrality was evaluated using the Ewens-Watterson test.

Results: According to the data of the Ewens-Watterson test for the neutrality of SNP-markers associated with psycho-neurological diseases, 10 loci showed a deviation from neutrality in 16 populations of North Eurasia: *CNTNAP2* rs10273775, *NCAPD3* rs1031381, *SNX29* rs12922317, *DCHS2* rs1466662, *CLU* rs1532278, *LSM1* rs16887244, *CD33* rs3826656, *ACSM1* rs433598, *PICALM* rs561655, *NECTIN2* rs6859. The maximum number of natural selection signals was noted for Tuvinians, Udmurts and Khants. Genetic marker rs1031381 at gene *NCAPD3* demonstrated deviation from selective neutrality in 10 populations of North Eurasia.

Conclusions: Natural selection contributes to the genetic diversity for schizophrenia and Alzheimer's disease genes in the North Eurasia populations. This work was supported by the Russian Foundation for Basic Research (project 18-29-13045).

A. Bocharova: None. **V. Stepanov:** None.

P19.056.D Evaluating the causal role of serum phosphate on bone mineral density: a Mendelian randomization study

Haotian Tang, *Jie Zheng, Tom R. Gaunt, Jonathan H. Tobias*

University of Bristol, Bristol, United Kingdom.

Rare genetic disorders leading to phosphate deficiency are associated with defective mineralization, but it is unclear whether serum phosphate is related to bone mineral density (BMD) in the wider population.

We conducted observational and Mendelian randomization (MR) analyses to evaluate the relationship between serum phosphate and BMD. Linear regression was used to examine associations between phosphate and BMD estimated from heel ultrasound (eBMD), adjusted for age, sex, BMI and albumin-corrected calcium, in 199,228 UK Biobank participants. In univariate MR analysis, instruments were single nucleotide polymorphisms (SNPs) associated with phosphate level in a GWAS of UK Biobank 431,510 participants. The outcome data were dual-energy x-ray absorptiometry (DXA)-based BMD at four body sites from the GEFOS consortium (gefos.org). Multivariable MR was conducted to estimate the independent effect of phosphate on BMD conditioning on calcium. To further identify causal genes linking phosphate with BMD, we conducted a gene-based MR by selecting eight phosphate associated genes (including α-klotho).

Observational analysis suggested a negative association between phosphate and eBMD ($\beta = -0.048$, 95%CI: -0.051~-0.044). Univariate MR showed weak evidence of a causal effect of phosphate on total body BMD ($\beta = 0.080$; SD change in total body BMD per SD change in phosphate; 95%CI: 0.001~0.160). However, multivariable MR showed a causal effect of phosphate on forearm BMD ($\beta = -0.140$; -0.277~-0.002), conditioning on calcium. Gene-based MR suggested that genetic signals of phosphate in α-klotho region were associated with forearm BMD. These results imply that increasing serum phosphate may cause decreased forearm BMD, which are less likely to reflect horizontal pleiotropy.

H. Tang: None. **J. Zheng:** None. **T.R. Gaunt:** None. **J.H. Tobias:** None.

P19.057.A The Trisomy 21 Prevalence in the Moscow Region of Russia

Nataliya Demikova[1,2], *Elizaveta E. Zayaeva*[2], *Marina Podolnaya*[1], *Aleksandra Lapina*[1], *Aliy Asanov*[3]

[1]*Research and Clinical Institute for Pediatrics named Academician Yuri Veltischev of the Pirogov Russian National Research Medical University of the Russian Ministry of Health, Moscow, Russian Federation,* [2]*Russian Medical Academy of Continuous Professional Education of the Ministry of Healthcare of the Russian Federation, Moscow, Russian Federation,* [3]*I.M. Sechenov First Moscow State Medical University, Moscow, Russian Federation.*

Trisomy 21 (T21) or Down syndrome is one of the most common chromosomal diseases. A well-known risk factor for having a child with T21 is the increased age of the mother. Since the 70s of the century, in Russia there has been an increase in the average maternal age. Monitoring the T21 prevalence is necessary due to changes in mothers age structure and the impact of preventive measures.

The aim: To analyze dynamics of T21 prevalence in the Moscow region - one of the largest regions of the Russian Federation for the period from 2011 to 2019.

Materials: The total number of newborns in the Moscow region from 2011 to 2019 was 771681; cases of T21, including newborns and eliminated fetuses, was 1490. The prevalence rate is calculated for 10,000 newborns.

Results: Total prevalence T21 in Moscow region was 19,31, while T21 prevalence among live born was 7,36. For the analyzed period, there is an increase in T21 total prevalence: from 18,30 in 2011 it increased to 23,33 in 2019. At the same time, T21 prevalence among live born decreased from 9,41 to 6,65 and the proportion of eliminated fetuses with T21 increased from 48,57% to 71,51%, which indicates the sufficient effectiveness of prenatal diagnosis.

Conclusion: In recent years T21 incidence rate tends to increase, one of the reasons for which is the increase in proportion of older women among pregnant women. At the same time, due to of prenatal screening, T21 prevalence in the Moscow region is decreasing.

N. Demikova: None. **E. Zayaeva:** None. **M. Podolnaya:** None. **A. Lapina:** None. **A. Asanov:** None.

P19.058.B Preliminary study of association of tuberculosis forms with polymorphisms in *IFN-γ, IL-1β, NOS2, MARCO* and *TLR8* genes

Ainur Akhmetova[1,2], *Ulan Kozhamkulov*[1], *Dauren Yerezhepov*[1], *Ainur Akilzhanova*[1,2]

[1]*Laboratory of Genomic and Personalized Medicine, National Laboratory Astana, Nazarbayev University, Nur-Sultan, Kazakhstan,* [2]*Department of General Biology and Genomics, L.N.Gumilyov Eurasian National University, Nur-Sultan, Kazakhstan.*

Introduction: Kazakhstan is one of 30 countries with high incidence of multi-drug resistant tuberculosis (MDR-TB) in the world. Various studies reveal association of polymorphisms of *IFN-γ, IL-1β, NOS2, MARCO* and *TLR8* genes with development of different forms of tuberculosis.Aim of this study is to evaluate association of *IFN-γ, IL-1β, NOS2, MARCO* and *TLR8* gene polymorphisms with development of different forms of tuberculosis (sensitive-TB, mono-resistant TB, poly-resistant TB, MDR-TB) in Kazakhstani patients.

Materials and Methods: 80 TB patients from 3 regions of Kazakhstan were involved in this research. Genotyping of samples was conducted on Applied Biosystems 7900HT using TaqMan probes rs2430561, rs16944, rs2274894, rs17009726 and rs3764880 for SNP markers of *IFN-γ, IL-1β, NOS2, MARCO* and *TLR8*, respectively. Drug resistance of *M. tuberculosis* isolates to first-line anti-TB drugs - isoniazid, rifampicin, ethambutol and streptomycin was determined by Sanger sequencing of *katG, rpoB, embB* and *rpsL* genes responsible for resistance to the drugs, respectively.

Results: Association of AT and AA genotypes of *IFN-γ* and *TLR8* with development of poly-resistant TB (85.7% and 57.1%, respectively); GA and AA genotypes of *IL-1β* and *NOS2* with development of MDR-TB (65.2% and 73.9%, respectively); AA genotype of *MARCO* with development of mono-resistant TB (100%) were detected. However, the obtained results were not statistically significant showing p-value 0.367, 0.786, 0.097, 0.458 and 0.314 respectively.

Conclusions: Association between polymorphisms of the genes and infection with different forms of tuberculosis were not identified in our research. In future, sample size should be increased to confirm the obtained results.

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P19.059.C Genome Diversity in Ukraine

Taras K. Oleksyk[1,2], *Khrystyna Shchubelka*[1,2], *Walter W. Wolfsberger*[1,2], *Yaroslava Hasynets*[2], *Olga T. Oleksyk*[3], *Volodymyr Smolanka*[2]

[1]*Oakland University, Rochester, MI, USA,* [2]*Uzhhorod National University, Uzhhorod, Ukraine,* [3]*A. Novak Transcarpathian Regional Clinical Hospital, Uzhhorod, Ukraine.*

The main goal of this collaborative effort is to provide genome wide data for the previously underrepresented population in Eastern Europe, and to provide cross-validation of the data from genome sequences and genotypes of the same individuals acquired by different technologies. We collected 97 genome-grade DNA samples from consented individuals representing major regions of Ukraine that were consented for the public data release. The genome data has been searched for genomic variation represented in this population, and a number of variants have been reported: large structural variants, indels, CNVs, SNPs and microsatellites. This study provides the largest to-date survey of genetic variation in Ukraine, creating a public reference resource aiming to provide data for historic and medical research in a large understudied population. While most of the common variation is shared with other European populations, this survey of population variation contributes a number of novel SNPs and structural variants that have not been reported in the gnomAD/1KG databases representing global distribution of genomic variation. These endemic variants will become a valuable resource for designing future population and clinical studies, help address questions about ancestry and admixture, and will fill a missing place in the puzzle characterizing human population diversity in Eastern Europe. Our results indicate that genetic diversity of the Ukrainian population is uniquely shaped by the evolutionary and demographic forces, and cannot be ignored in the future genetic and biomedical studies.

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P19.060.D Thrombosis during pregnancy and postpartum period in Georgian women with inherited thrombophilia: comparative analysis

Ketevan Kartvelishvili[1,2], *Nino Pirtskhelani*[1,2], *Nino Kochiashvili*[2], *Levan Makhaldiani*[3]

[1]*Tbilisi State Medical University, Tbilisi, Georgia,* [2]*Levan Samkharauli National Forensics Bureau, Tbilisi, Georgia,* [3]*National Surgery Center's Clinic "Akhali Sitsotskhle", Tbilisi, Georgia.*

Introduction: Venous Thromboembolism (VTE) is one of the leading causes of maternal mortality. The increased risk of thrombosis first appears in the beginning of pregnancy and reaches its maximum in the postpartum period. About 50% of pregnancy-associated VTE occurs during pregnancy itself and 50% in the "critical period" within six weeks after delivery; thus the risk of postpartum thrombosis is about 5 times higher than during pregnancy itself. Hereditary thrombophilia increases the risk of pregnancy associated VTE up to 34-fold.

Aim: The aim of this study was to analyze maternal VTE during pregnancy and postpartum period in Georgian women with inherited thrombophilia.

Materials and Methods: 421 Georgian women with pregnancy complications (Miscarriages, stillbirth, VTE and etc.) were investigated for detection of FV Leiden, prothrombin G20210A, MTHFR C677T mutations by PCR analysis.

Results: Out of 421 women 43(10.22%) had VTE, 17(4.04%) during pregnancy and 26(6.18%) postpartum. Out of 17 patients with VTE during pregnancy, thrombophilia gene mutations were detected in 6(35.39%) cases (FVL-4, Pr-3, MTHFR-1), Compare to 26 patients with VTE during postpartum period - in 15(57.69%) cases (FVL-9, Pr-5, MTHFR-2). The combined double and triple mutations were detected in 3 cases. Family history of thrombosis was positive in 13(76.47%) patients with VTE during pregnancy, in 19 (73.08%) patients with VTE during postpartum period.

Conclusion: According to our data, significant prevalence of VTE in association with thrombophilia was detected in postpartum period, compare to VTE during pregnancy. These data resembles the results of other populations.

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P19.061.A Investigating the origin of the Ottoman dynasty based on Y Haplogroup-related markers

Zsolt Bánfai[1,2], *Attila Gyenesei*[2], *Judit Bene*[1,2], *Norbert Pap*[3], *Attila Miseta*[4], *Miklós Kásler*[5], *Béla Melegh*[1,2]

[1]*Department of Medical Genetics, University of Pécs, Pécs, Hungary,* [2]*Szentágothai Research Centre, University of Pécs, Pécs, Hungary,* [3]*Department of Political Geography, Development and Regional Studies, Faculty of Sciences, University of Pécs, Pécs, Hungary,* [4]*Department of Laboratory Medicine, Medical School, University of Pécs, Pécs, Hungary,* [5]*National Institute of Oncology, Budapest, Hungary.*

Introduction: The four most common Y chromosome haplogroups in Turkey are J2, R1b, G and E3b. R1a is the sixth most common haplogroup among the male members of the Turkish population. The actual haplogroup of the House of Osman is still controversial, the Ottoman dynasty might belong either the J2, or the R1a haplogroup. The J2 haplogroup is supported by the conventional analysis of a known recent descendant on the direct male line of H.I.H. Prince (Şehzade) Yusuf Izzeddin (1857-1916), the first-born son of Sultan Abdülaziz (1830-1876) and the older brother of Abdulmejid II (1868-1944). This test revealed the J2a (J-M410) haplogroup.

Materials and Methods: Whole genome sequencing was performed on a DNA sample belonging to a male descendant of the Ottoman dynasty, H.I.H. Prince (Şehzade) Mahmud Namık Osmanoğlu, a member of the direct male lineage of Sultan Mehmed V Reşâd (1844-1918). The BCFtools package was used for variant calling and the AMY-tree algorithm was applied to determine the relevant set of Y haplogroup markers.

Results: The analysis identified 84 markers and indicated that the sample belongs to the J2a2 (J-L581) haplogroup.

Conclusion: Results revealed that the J2 is the common haplogroup of the Ottoman dynasty. This haplogroup is known to be common on the Anatolian peninsula and can be found also in the Caucasus region and in Central Asia. Relationship of these pools should be further investigated.

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P19.062.B The Dutch Y-chromosomal landscape from theEarly Middle Agesto present day

Eveline Altena[1], *Risha Smeding*[1], *Eileen Vaske*[1], *Paul Reusink*[1], *Oscar Lao*[2,3,4], *Kristiaan J. van der Gaag*[1,5], *Rick H. de Leeuw*[1], *Thirsa Kraaijenbrink*[1], *Yoan Diekmann*[6], *Manfred Kayser*[2], *Mark G. Thomas*[6], *Peter de Knijff*[1]

[1]*Leiden University Medical Center, Leiden, Netherlands,* [2]*Erasmus MC University Medical Center Rotterdam, Rotterdam, Netherlands,* [3]*Barcelona Institute of Science and Technology, Barcelona, Spain,* [4]*Universitat Pompeu Fabra, Barcelona, Spain,* [5]*Netherlands Forensic Institute, The Hague, Netherlands,* [6]*University College London, London, United Kingdom.*

Epidemiological, forensic and historical studies can greatly benefit from detailed information on historical and present-day geo-genetic patterns and population continuity. We present this information for the Netherlands for Y-chromosomal SNPs based on an historical dataset of over 300 early and late medieval and Early Modern Period males and a present-day dataset of over 2000 males. The historical samples were collected from excavations at 13 locations, the present-day dataset contains samples from 99 locations, evenly spread across the country. Using different methods we observed statistically significant differentiation between periods in general, between locations within both the historical periods and present day and between periods within locations. We could, however, not reject population continuity, which is relevant to future (paleo)epidemiological and selection studies in the datasets presented here. Visualization of genetic distances between locations and periods indicated a decrease of reduced genetic distance over time between locations but was found to not be statistically significant with formal testing. In conclusion, the changes in geo-genetic patterns for the Y-chromosome in the Netherlands from the Early Middle Ages to present day indicate that the modern patterns formed recently. Since we cannot reject population continuity, drift needs to be considered as a key factor in these changes, besides demographic events. We should therefore be careful to assign observations in present-day data to specific historical events.

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P19.063.C New insights into the evolution of a human Y chromosome singleton palindrome

Maria Bonito[1], *Eugenia D'Atanasio*[2], *Francesco Ravasini*[1], *Selene Cariati*[1], *Biancamaria Bonucci*[1], *Andrea Finocchio*[3], *Andrea Novelletto*[3], *Fulvio Cruciani*[1,2,4], *Beniamino Trombetta*[1]

[1]*Department of Biology and Biotechnology "Charles Darwin", Sapienza University of Rome, Rome, Italy,* [2]*Institute of Molecular Biology and Pathology, CNR, Rome, Italy,* [3]*Department of Biology, University of Rome Tor Vergata, Rome, Italy,* [4]*Istituto Pasteur - Fondazione Cenci Bolognetti, Rome, Italy.*

Introduction: About 25% of the euchromatic portion of the male-specific region of the human Y chromosome consists of large duplicated sequences, organized in eight palindromes (P1-P8) which undergo arm-to-arm gene conversion, a proposed mechanism for maintaining their sequence integrity. Despite the relevance of gene conversion in palindrome evolution, its dynamic is still nuanced.

Materials and Methods: We reanalysed the genetic diversity of 3.3 Mb of the X-degenerate region in 157 Y chromosomes to reconstruct an unambiguous phylogeny. Subsequently, for the same samples we performed a high depth (>50×) targeted NGS of P6, the largest Y chromosome singleton palindrome.

Results: We identified 118 new paralogous sequence variants and 80 gene conversion events that shaped the diversity of P6 arms during recent human history. We also estimated a Y-Y gene conversion rate of 6.01×10^{-6} conversions/base/year.

Conclusions: We found that: 1) Y-Y gene conversion is not biased towards the ancestral state, 2) the establishment of a mutation/conversion balance drives the evolution of P6 arms, 3) gene conversion, in spite of maintaining the palindrome structural integrity, can be involved in the loss of genetic material from the arms, 4) the higher mutation rate of the spacer compared to the arms may explain the observed lower divergence between the orthologous duplicated sequences of the palindrome compared to its haploid portion, without invoking any conversion bias. "Programmi di Ricerca 2018-2020", Istituto Pasteur—Fondazione

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P20 Functional Genomics and Epigenomics

P20.001.D Investigating the meaning of age-related changes in DNA methylation by studying the correlation between epigenetic age acceleration and progressive human appearance traits

Rezvan Noroozi[1], *Aleksandra Pisarek*[1], *Joanna Rudnicka*[1], *Michał Boroń*[2], *Kamila Migacz-Gruszka*[3], *EPIGENOME Consortium, Aneta Sitek*[4], *Andrzej Ossowski*[5], *Wojciech Branicki*[1,2], *Magdalena Spólnicka*[2], *Ewelina Pośpiech*[1]

[1]*Malopolska Centre of Biotechnology, Jagiellonian University, Kraków, Poland,* [2]*Central Forensic Laboratory of the Police, Warsaw, Poland,* [3]*Department of Dermatology, Collegium Medicum of the Jagiellonian University, Kraków, Poland,* [4]*Department of Anthropology, Faculty of Biology and Environmental Protection, University of Łódź, Łódź, Poland,* [5]*Department of Forensic Genetics, Pomeranian Medical University in Szczecin, Szczecin, Poland.*

Introduction: DNA methylation markers have been proposed as a predictor of biological age. At the same time, phenotypic aging is a potential model for exploring the molecular mechanisms of aging. By investigating the correlation between epigenetic age acceleration (EAA) and externally visible phenotypes we aim to investigate molecular pathways involved in aging processes and disclose promising targets for antiaging therapies.

Materials and Methods: A cohort of about 1000 individuals of European descent with described physical phenotype and collected lifestyle information will be examined using Infinium® Global Screening Array and Infinium® MethylationEPIC 850K microarray. DNA methylation data will be examined using various age prediction models to calculate EAA. The EAA values will be further correlated with phenotypic traits including hair loss, hair greying, and wrinkles formation as well as with genetic variation.

Results and Conclusion: The study will improve our understanding of the role of interactions between genes, DNA methylation, and EAA in determining age-related appearance traits. We will assess the heritability of the aging rate and measure the importance of environmental factors for accelerated aging. The role of individual CpG and SNP markers will be tracked in enrichment analysis. The study will have practical value in forensics by developing prototype predictive models for specific age-related features based on genetic and epigenetic information, as well as may find practical application in the cosmetic industry by developing products to prevent or slow down phenotypic aging. This research was supported by the grant from the National Centre for Research and Development no DOB-BIO10/06/01/2019.

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P20.002.A A multi-omics approach to study monozygotic twins discordant for amyotrophic lateral sclerosis

Martina Tosi[1], *Miriam Zuccalà*[1], *Francesco Favero*[1], *Lucia Corrado*[1], *Roberta Croce*[1], *Chiara Basagni*[1], *Nadia Barizzone*[1], *Laura Follia*[2], *Fabiola De Marchi*[3], *Elena Chinni*[4], *Letizia Mazzini*[3], *Davide Corà*[1], *Maurizio Leone*[4], *Sandra D'Alfonso*[1]

[1]*University of Eastern Piedmont UPO, Novara, Italy,* [2]*University of Torino, Torino, Italy,* [3]*ALS Center AOU Maggiore della Carità, Novara, Italy,* [4]*SC Neurologia, Dipartimento di Scienze Mediche, IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Foggia, Italy.*

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease, characterised by progressive death of upper and lower motor neurons. The majority of ALS cases are sporadic, while 10% are familial. ALS aetiology is still not completely understood. To investigate genetic and epigenetic factors underlying ALS, we studied a monozygotic twin pair discordant for ALS with a multi-omics approach combining whole exome sequencing with genome-wide methylome- and transcriptome data from whole blood and PBMCs. For methylation, we used the Illumina EPICArray which covers 850,000 methylations sites and the ChaAMP software for the analyses, while for gene expression study, Illumina TruSeq Stranded mRNA sequencing was performed. The results were considered independently and in combination to identify disease-relevant methylation changes and their downstream impact. Twins tested negative for mutations in main ALS-genes. We identified 59 differentially expressed genes (DEGs) (p.adj < 0.1; |log2FC| >1) involved in immune system pathways. After QC, we found 2 differentially methylated probes (pvalue adj ≤ 0.1) in CACNA1G, expressed mostly in brain, and VAX1 genes; while filtering by delta beta (Δβ) values, we identified 2 probes with Δβ ≤ - 0.25 (in an intergenic region and RUSC1-AS1) and 2 probes with Δβ ≥ 0.25 (in AARS and KPNA4). None of them fell into the highlighted DEGs. Finally, mRNA-seq results were compared with larger literature datasets. Further comparative analyses on external epigenetic datasets as well as CNV and SNV analyses on exome data are ongoing to elucidate possible epigenetic and somatic genetic factors that could underlie susceptibility to sporadic ALS.

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P20.003.B Resistance profile and genetic diversity among selected ESBL-producing *Escherichia coli* isolates from urocultures in a portuguese hospital

Isabel Carvalho[1,2,3,4,5], *José António António Carvalho*[6], *Ana Paula Castro*[6], *Sandra Martínez-Álvarez*[5], *Gilberto Igrejas*[2,3,4], *Carmen Torres*[5], *Patrícia Poeta*[1,4]

[1]*Microbiology and Antibiotic Resistance Team (MicroART), Department of Veterinary Sciences, University of Trás-os-Montes and Alto-Douro), Vila Real, Portugal,* [2]*Department of Genetics and Biotechnology, UTAD, Vila Real, Portugal,* [3]*Functional Genomics and Proteomics Unit, UTAD, Vila Real, Portugal,* [4]*Laboratory Associated for Green Chemistry (LAQV-REQUIMTE), New University of Lisbon, Monte da Caparica, Portugal,* [5]*Area Biochemistry and Molecular Biology, University of La Rioja, Logroño, Spain,* [6]*Medical Center of Trás-os-Montes e Alto Douro E.P.E., Vila Real, Portugal.*

Introduction: Antimicrobial-resistant bacteria are contributing to mortality and morbidity worldwide. The Extended-Spectrum β-lactamase-producing *E. coli* is considered one of the great concerns regarding the public health issue. The purpose of this work was to determine prevalence and genetic characteristics of selected ESBL-producing *E. coli* isolates from urinary infections.

Materials and Methods: Twenty cefotaxime/ceftazidime-resistant *E. coli* isolates were obtained aleatory from urocultures in a Portuguese hospital, during June 2017-July 2018. Identification was performed by MALDI-TOF MS. Antimicrobial susceptibility

against 13 antibiotics was analyzed by disk diffusion test. Screening of ESBLs was performed and resistance genes were analyzed by PCR/sequencing. Phylogenetic grouping was also performed by multiplex-PCR.

Results: ESBL-production was detected in 90% of the isolates (18/20), mostly associated with CTX-M-15 (n = 13) and CTX-M-1 (n = 1) enzymes. Tetracycline resistance was associated with *tet*A (n = 5) and *tet*B (n = 3). The most common phylogenetic group among ESBL-producers was B2 (n = 13), followed by D (n = 2), C (n = 1) and A (n = 1). The isolates carrying the $bla_{\text{CTX-M-15}}$ gene were ascribed to phylogroups B2 and D, and the $bla_{\text{CTX-M-1}}$-carrying isolate was typed as phylogroup C. The two ESBL-negative *E. coli* isolates also carried a CTX-M gene (which variant was not determined).

Conclusions: These findings indicate that the CTX-M-15 enzyme is the main mechanism of ESBL-production among urinary infections isolates in our hospital, being these isolates of the phylogroups B2 and D.

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P20.004.C E6 and E7 HPV16 oncogenes influence gene transcription trough the genome-wide pattern deposition of MBD2,3 components of NuRD nucleosome remodeling complex

***Anca Botezatu**, Adriana Plesa, Iulia V. Iancu, Marinela Bostan, Mirela Mihaila, Adrian Albulescu, Alina fudulu, Carmen C. Diaconu, Gabriela Anton*

Instute of Virology "St.S.Nicolau", Bucharest, Romania.

Human papilloma virus (HPV) is the etiologic agent of cervical cancer and the third most commonly diagnosed type of cancer in women worldwide. The nucleosome remodelling and deacetylation complex (NuRD) is a group of associated proteins with ATP-dependent chromatin remodelling and histone deacetylase activities. MBD2/3 proteins from NuRD complex exhibit methyl-CpG-binding domains, which mediate an interaction with methylated DNA. The current study aims to assess the viral oncogenes influence on the MBDs overall binding pattern to CpG islands. ChIP-Seq for MBD2/3 genome wide DNA binding pattern (e.g. promoters, gene control region, transcriptional enhancers, etc.) in untreated and HPV 16 E6/E7 shRNAs treated CaSki cell culture was performed and the results were analysed using Base Space Illumina apps. MBD2/3 proteins were localized at the level of intron, intergenic regions and TSS. After ChIP-Seq peak score analysis, a cut-off of 9 was establish and 54 gene loci were identified. The corresponding genes were further analysed in qRT-PCR and their expression was found to be deregulated. When both oncogenes (E6 and E7) were silenced, we noted an enrichment of MBD2/3 proteins at CDK6, DLC1, NRIP1 gene loci involved in oestrogen receptor (ER) signalling pathway. Another interesting gene loci involved in mRNA processing and cancer growth and metastasis were identified (EIF4G3 and DCP2). Viral oncogenes act synergistically on the gene transcription pattern by interacting with the MBD2/3 proteins of NuRD complex. Epigenetic gene control is a complex phenomenon that is guided by internal, cellular and external factors as well as viral infections. **Acknowledgments: TE39/2020**

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P20.005.D New *cis*-regulatory elements modulate *CFTR* expression

***Mégane COLLOBERT**[1], Ozvan Bocher[1], Anaïs Le Nabec[1], Emmanuelle Génin[2], Claude Férec[1,3], Stéphanie Moisan[1,3]*

[1]Univ Brest, Inserm, EFS, UMR 1078, GGB, Brest, France, [2]Inserm, Univ Brest, EFS, UMR 1078, GGB, Brest, France, [3]Univ Brest, CHRU Brest, UMR 1078, Brest, France.

8% of the human genome is covered with candidate *cis*-regulatory elements (cCREs). Anomalies of CREs at distance from a gene have been identified as being involved in various genetic diseases. Although, more than 2100 variants have been detected in *Cystic Fibrosis Transmembrane conductance Regulator* (*CFTR*) gene, responsible of Cystic Fibrosis (CF) or *CFTR*-related disorders, some patients have an incomplete genotype or present extremes phenotypes. Development of chromatin conformation study techniques identified several long-range regulatory elements of *CFTR* gene. Our aim is to highlight the role and involvement of regulatory elements on the architecture and conformation of the *CFTR* gene.GWAS3D score application allowed us to highlight involvement of four *CFTR* introns in gene regulation, introns 26 (4374 + 1,3 kb), 24 (4095 + 7,2 kb), 1 (185 + 10 kb) et 12 (1811 + 0,8 kb). Introns 1 and 12 have already been described as two main cooperative *CFTR* CREs in intestinal cells. Activity tests in Caco-2 intestinal cells show strong cooperative effects of the four predicted introns on *CFTR* promoter activity. Chromatin immunoprecipitation analyses demonstrate enrichment of a large network of keys transcription factors (TFs) in intestinal cells, such HNF1a, p300, FOXA1/A2, CDX2 and TCF4, in introns 24 and 26 enhancers. In conclusion, two new CREs with cooperative enhancer activities have been identified, enriched with important TFs, redefining the 3D regulation model of the *CFTR* gene in intestinal cells. Ongoing studies of chromatin conformation and CRISPR interference will further characterize the role of these new enhancers.

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P20.006.A Functional characterisation of *GJB2 cis* regulatory elements and WGS of heterozygous patients with NSHL

***Anaïs LE NABEC**[1], Mégane Collobert[1], Alicia Quillévéré[1], Cédric Le Maréchal[1,2], Claude Férec[1,2], Stéphanie Moisan[1,2]*

[1]Univ Brest, Inserm, EFS, UMR 1078, GGB, brest, France, [2]Univ Brest, CHRU Brest, UMR 1078, Brest, France.

Three-dimensional chromatin organization plays a key role on gene expression. Gene regulation depends on *cis*-regulatory elements which can interact with gene promoter by chromatin loop. Alteration of chromatin architecture and/or *cis*-acting elements can lead to *enhanceropathies*. Several unelucidated nonsyndromic hearing loss and deafness 1 (*DFNB1)* cases carrying out only one heterozygous pathogenic mutation on Gap Junction Beta 2 (*GJB2*) gene, led to strongly suggest the presence of distant *cis*-regulation. Thanks to chromatin conformation study, we previously identified several *cis*-regulatory elements which have enhancer action and silencer effect on *GJB2* expression. Analysis of CTCF binding allowed to purpose a *DFNB1* 3D looping model. We identified cooperative effects between enhancers. To confirm an

endogenous enhancer activity, we develop CRISPR interference (CRISPRi), new approach for targeted silencing of transcription in human cells. We target *GJB2 cis*-acting elements with dCas9-KRAB. Preliminary results show a decrease of *GJB2* expression. Then, we focus on 10 patients with incomplete genotype. We realize a whole genome sequencing with HiSeq 4000 by IntegraGen Genomics. WGS analysis allowed to redress three genotype. Moreover, we realize functional assays to analyse a variant in *GJB2* promoter and continue analyses for the others genotypes. This work is supported by grants from the French foundation "La Fondation pour l'Audition", the "Région Bretagne" and the association "Gaétan Salaün".

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P20.007.B The level of myeloperoxidase gene methylation in peripheral blood leukocytes is an epigenetic marker of coronary artery disease

Olga Bushueva[1], *Ekaterina Barysheva*[2], *Anton Markov*[3], *Iuliia Koroleva*[3], *Egor Churkin*[3], *Vladislav Soldatov*[4], *Alexey Polonikov*[1], *Vladimir Ivanov*[2], *Maria Nazarenko*[3]

[1]*Research Institute for Genetic and Molecular Epidemiology, Kursk State Medical University, Kursk, Russian Federation,* [2]*Department of Biology, Medical Genetics and Ecology, Kursk State Medical University, Kursk, Russian Federation,* [3]*Institute of Medical Genetics, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russian Federation,* [4]*Department of Pharmacology and Clinical Pharmacology, Institute of medicine, Belgorod State National Research University, Belgorod, Russian Federation.*

Introduction: Coronary artery disease (CAD) is the leading cause of death worldwide. Despite the evidence for the role of oxidative stress in the development of CAD, there are few studies on site-specific DNA methylation of genes involved in the regulation of vascular redox homeostasis. We aimed to analyze a possible association of DNA methylation levels of oxidative-stress-related genes with CAD.

Materials and Methods: DNA methylation patterns in the promoter or regulatory regions of 4 genes (*GCLM*, *GSTP1*, *TXNRD1*, and *MPO*) in peripheral blood leukocytes of 45 patients with CAD and in 83 sex- and age-matched healthy controls were analysed. Quantitative DNA methylation analysis of the bisulfite-treated DNA was performed by pyrosequencing on a PyroMark Q24 (Qiagen, Germany).

Results: Statistically significantly lower methylation levels were registered at a CpG site (chr1:94374293, GRCh37 [hg19]) in *GCLM* in patients with CAD compared with the control group (6,1% [4,8%; 7.6%] (median and interquartile range) versus 14.5% [10.4%; 21,7%], respectively, $p = 1.49 \times 10^{-11}$). The most pronounced differences between the patients and controls were uncovered in the analysis of myeloperoxidase gene methylation. In the leukocytes of patients with CAD, the methylation level of CpG sites in the analyzed region of *MPO* (chr17:56356470, GRCh3 [hg19]) on average was significantly lower (26.5% [24.5%; 32.3%]) than that in the control group (35.4% [30.3%; 42.6%], $p = 3.83 \times 10^{-7}$).

Conclusions: Thus, hypomethylation of CpG sites in *MPO* in blood leukocytes can be considered a diagnostically significant epigenetic marker of coronary artery disease. Further epigenetic studies of the oxidative-stress-related genes in CAD are required.

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P20.009.D Polymorphism of methyl group metabolism gene as a modifier of Cystic Fibrosis phenotype

Eka Kvaratskhelia[1,2], *Tinatin Tkemaladze*[1], *Mariam Ghughunishvili*[3], *Maia Gagua*[2], *Sandro Surmava*[1], *Maia Zarandia*[1], *Elene Abzianidze*[1]

[1]*Department of Molecular and Medical Genetics, Tbilisi State Medical University, Tbilisi, Georgia,* [2]*Bakhutashvili Institute of Medical Biotechnology, Tbilisi State Medical University, Tbilisi, Georgia,* [3]*Tbilisi State Medical University, G.Zhvania Pediatric Academic Clinic, Tbilisi, Georgia.*

Introduction: Cystic fibrosis (CF) is a common, life-limiting monogenic disease, which typically manifests as progressive bronchiectasis, exocrine pancreatic dysfunction, and recurrent pulmonary infections. Modifier genes and epigenetic factors play important roles in determining the severity of disease. Identifying these factors is crucial in personalized treatment approaches. In a previous study, we demonstrated that global DNA methylation was significantly decreased in CF individuals with *MTHFR* gene T677C variant. In this study we analyzed phenotypes of CF individuals with TT and CC genotypes of *MTHFR* gene.

Methods: The study was approved by the ethical committee of the TSMU. We selected CF patients homozygous or compound heterozygous for *CFTR* mutations. We analyzed *MTHFR* gene using PCR-RELP method. In 4 patients *with MTHFR TT* genotypes we analyzed severity of CF and compared with 4 patients CC genotypes.

Results: We observed that three CF patients (homozygotes or compound heterozygous for a class I-II) with TT genotypes had failure to thrive, chronic bronchopulmonary infection, bronchiectasis and pancreatic insufficiency. Four subjects homozygotes or compound heterozygous for a class I-II mutations of CFTR gene and with *MTHFR* CC genotypes had a less severe phenotypic expression with milder lung inflammation without pancreatic insufficiency.

Conclusion: There is strong correlation between the general type of *CFTR* mutation and clinical phenotype. However, among patients carrying two mutations with no residual function, there is also a very broad range of lung disease severity maybe due to modifier genes involved in methyl group metabolism.

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P20.010.A Genetic and epigenetic alterations in Pituitary Neuroendocrine Tumors (PitNETS) with different clinical outcome: the role of X-linked genes

Luca Morandi[1,2], *Stefania Evangelisti*[1], *Caterina Tonon*[1,2], *Matteo Zoli*[3], *Diego Mazzatenta*[3], *Federica Guaraldi*[4], *Alberto Righi*[5], *Sofia Asioli*[6]

[1]*Department of Biomedical and Neuromotor Sciences, University of Bologna, Functional and Molecular Neuroimaging Unit, IRCCS Istituto delle Scienze Neurologiche di Bologna, Bologna, Italy,* [2]*IRCCS Istituto delle Scienze Neurologiche di Bologna, Italy, Bologna, Italy,* [3]*Department of Biomedical and Neuromotor Sciences, University of Bologna, Pituitary Unit, IRCCS Istituto Delle Scienze Neurologiche di Bologna, Bologna, Italy,* [4]*Pituitary Unit, IRCCS Istituto Delle Scienze Neurologiche di Bologna, Bologna, Italy,* [5]*Service of Anatomic Pathology, IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy,* [6]*Department of Biomedical and Neuromotor Sciences, University of Bologna, Unit of Anatomic Pathology "M. Malpighi", Bellaria Hospital, Bologna, Italy.*

Introduction: Pituitary neuroendocrine tumours(PitNETS) can show an aggressive clinical behaviour presenting local invasion, post-surgical recurrence and resistance to treatment. This study aimed to identify novel biomarkers of prognosis and postsurgical outcome.

Materials and Methods: 59 non recurrent, 17 recurrent PitNETS and 5 pituitary carcinomas were investigated for mutation and DNA methylation analysis in 15 and 32 driver genes respectively.

Results: 9/59(15.3%) non recurrent and 10/17 recurrent(58.8%) PitNETS showed at least one mutation. *TP53*(13/76), *NOTCH1*(6/76) and *EGFR*(4/76) are the most frequently mutated genes. An increasing trend of DNA methylation was detected starting from normal tissue, through non recurrent adenomas, recurrent PitNETs to carcinoma in *PARP15* and *MIR193a*. *PDCD1* and *AIP* showed the highest levels in non recurrent PitNETs, an intermediate level in recurrent PitNETS and lower levels in carcinoma. X-linked genes were analysed differentiating males and females: in females, *MAGEA2, MAGEA3, MAGEA4, UXT* and *FLNA* displayed the highest methylation levels in non recurrent PitNETS, lower levels in recurrent PitNETS and no methylation in carcinoma. *MAGEA11* showed the opposite behaviour. In males, carcinomas were found hypomethylated in *MAGEA1, MAGEA11* and *FLNA*, while *UXT* and *MAGEC1* were hypermethylated.

Conclusions: pituitary carcinoma, recurrent and non recurrent PitNETS can be stratified by *MIR193a, PARP15, PDCD1* and *AIP*. X-linked genes belonging to *MAGEA* family, *FLNA* and *UXT* showed a different methylation pattern depending on the gender. The combination of epigenetic and somatic profiling allows for the identification of a subset of more aggressive PitNETs that should be useful for prognostic stratification.

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P20.011.B Disease interpretation of non-coding genomic elements with the GeneCards Suite

Ruth Barshir[1], *Simon Fishilevich*[1], *Tsippi Iny-Stein*[1], *Ofer Zelig*[2], *Yaron Guan-Golan*[2], *Marilyn Safran*[1], *Doron Lancet*[1]

[1]*Weizmann Institute of Science, Rehovot, Israel,* [2]*LifeMap Sciences Inc, Alameda, CA, USA.*

Interpreting whole genome sequencing (WGS) data is a major challenge in genetics, since 98% of variants reside in non-coding genomic "dark matter" which includes regulatory elements, introns, untranslated regions, and non-coding RNAs (ncRNAs).

The GeneCards® Suite (https://www.genecards.org/) encompasses ~270k annotated coding and non-coding genes in GeneCards (PMID:27322403), and ~20k annotated diseases in MalaCards (PMID:27899610). VarElect (PMID:27357693), the Suite's NGS phenotype interpreter, leverages this knowledgebase to prioritize associations between genes and phenotype terms. We've made significant strides towards optimizing our Suite for effective interpretation of non-coding variants.

GeneHancer (PMID:28605766) is a database of regulatory elements encompassing 400k enhancers and promoters, covering 18% of the genome, and annotated with functional information, including accurate genomic coordinates, target genes, and tissue activity patterns. It integrates information from key epigenetic resources and is included as a native regulation track at the UCSC genome browser.

GeneCaRNA (Barshir et al, under review) is a novel gene-centric ncRNA database, integrating data from RNAcentral, a comprehensive ncRNA transcript database with 20 expert databases, and from the major gene resources HGNC, Ensembl and NCBI Gene. GeneCaRNA provides a comprehensive non-redundant view of 220k human ncRNAs of 17 functionally diverse types such as lncRNAs and miRNAs.

Our novel non-coding compendia GeneHancer and GeneCaRNA provide an indispensable augmentation for VarElect, powering the prioritization of variant-containing enhancers, promoters and ncRNAs with respect to diseases via direct and target-gene mediated links. These capabilities facilitate deciphering the clinical significance of non-coding variants identified by WGS, often elucidating unsolved clinical cases (PMID:32506582).

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P20.012.C Lifestyle-dependent epigenetic signatures and the impact of lifestyle on epigenetic age acceleration

Joanna Rudnicka[1], *Rezvan Noorozi*[1], *Aleksandra Pisarek*[1], *Michał Boroń*[2], *Aleksander Masny*[2], *Bożena Woźniak*[2], *Kamila Migacz-Gruszka*[3], *EPIGENOME Consortium, Aneta Sitek*[4], *Andrzej Ossowski*[5], *Wojciech Branicki*[1,2], *Magdalena Spólnicka*[2], *Ewelina Pośpiech*[1]

[1]*Jagiellonian University, Kraków, Poland,* [2]*Central Forensic Laboratory of the Police, Warszawa, Poland,* [3]*Department of Dermatology, Collegium Medicum of the Jagiellonian University, Kraków, Poland,* [4]*Department of Anthropology, Faculty of Biology and Environmental Protection, University of Łódź, Łódź, Poland,* [5]*Department of Forensic Genetics, Pomeranian Medical University, Szczecin, Poland.*

Introduction: Research shows that lifestyle influences the human epigenome by altering DNA methylation patterns. In forensics, reliable prediction of lifestyle from DNA traces can be informative in characterizing an unknown donor of a forensic specimen, and thus useful in guiding an investigation. Changes in DNA methylation patterns can also be signatures of habit-related diseases, and their study is of medical significance. Aim: The goal of the EPIGENOME project is to identify differentially methylated regions (DMRs) for selected habits factors including diet, physical activity and stressful experiences. An additional goal is to analyze selected CpGs associated with particular habits in terms of their correlation with aging processes and then validate them as markers for lifestyle-induced diseases.

Materials and methods: A set of 600 blood samples was collected from individuals aged above 30 years including groups exposed to extreme factors such as soldiers and professional bodybuilders. The project involves the extension of the list to the number of 800 blood samples. Each sample donor provided a detailed questionnaire. For the whole cohort genome- and epigenome data will be collected using the microarray technology (Illumina). The obtained SNP and methylation data will be used in statistical analyses.

Conclusions: The project will allow us to develop DNA methylation-based models for the prediction of individual's habits based on forensic material. The obtained data can also help shape the right habits for healthy aging. This research is supported by the grant from the National Centre for Research and Development no DOB-BIO10/06/01/2019.

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P20.014.A Functional characterization of variants in the 5UTR and promoter of *PCK9* gene

Ana Catarina Alves, *Juliane Menezes, Rafael Fernandes, Luísa Romão, Mafalda Bourbon*

Instituto Nacional de Saúde, Lisboa, Portugal.

Familial hypercholesterolemia (FH) is the most common genetic disorder conferring an increased cardiovascular risk due to cholesterol accumulation since birth. FH patients have usually mutations in *LDLR*, *APOB* or *PCSK9* genes, but in about 50% a variant causing disease is not identified. The 5′ and 3′ untranslated regions (UTRs) and promoter of these genes is poorly studied. The aim of this project is to define the *PCSK9* 5′UTR sequence and perform an in vitro characterization of variants in 5′ UTR and promoter of *PCSK9* gene. To define the *PCSK9* 5′UTR sequence we used a 5′-RACE kit that involved several steps. The promoter and 5′UTR regions of *PCSK9* (-650 to -1) was cloned into the pGL4.10 [luc2] plasmid containing Firefly luciferase. This construct was subjected to site-directed mutagenesis to obtain the variants under study. All the variants were transfected into HepG2 and luciferase activity was determined using the Dual-Luciferase Reporter Assay System in a GloMax Luminometer. We verified that the promoter in PCSK9 was concordant with that described in ENSEMBL. A total of 17 variants in the promoter region of the *PCSK9* gene described in ClinVar have been studied or are under study. Preliminary results suggest that 2 of the variants appear to be gain-of-function and 6 loss-of-function variants. Our results emphasize the necessity of functional analysis of new variants in these regions with the objective of determining their biological effect and possible influence on FH phenotype, allowing the correct diagnosis of the disease.

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P20.015.B Functional characterization of three GLYAT variants and the effect on phase II glycine conjugation

Rencia van der Sluis[1], *Johann Rohwer*[2], *Chantelle Schutte*[1]

[1]*North-West University, Potchefstroom, South Africa,* [2]*Stellenbosch University, Stellenbosch, South Africa.*

The glycine conjugation pathway is involved in the metabolism of natural substrates as well as the detoxification of xenobiotics. The interactions between the various substrates in this pathway and their competition for the pathway enzymes are currently unknown. The pathway consists of a mitochondrial xenobiotic/medium chain fatty acid: CoA ligase (ACSM2B) and glycine N-acyltransferase (GLYAT). In this study, the level of evolutionary conservation of the *GLYAT* gene was analysed and haplotype variants were selected (S_{156}, $T_{17}S_{156}$ and $S_{156}C_{199}$) in order to characterise the kinetic mechanism of the enzyme over a wide range of substrate concentrations. Cooperative substrate binding was observed and the kinetic data were fitted to a two-substrate Hill equation. The coding region of the *GLYAT* gene was found to be highly conserved and the rare $S_{156}C_{199}$ variant negatively affected the relative enzyme activity and k_{cat} parameter. The $S_{156}C_{199}$ variant displayed only 10% of the activity of the most abundant S_{156} haplotype, while the activity of $T_{17}S_{156}$ was 73% of S_{156}. The in vitro kinetic analyses indicated that individuals with the $S_{156}C_{199}$ haplotype might have a decreased ability to metabolise benzoate when compared to individuals with the S_{156} haplotype. This is due to the fact that benzoyl-CoA remains tightly bound to the enzyme and that binding of glycine (the second substrate) further decreases the affinity of the enzyme for benzoyl-CoA. Accumulation of acyl-CoA intermediates can inhibit ACSM2B leading to a reduction in mitochondrial energy production.Funding: National Research Foundation of South Africa (NRF), Grant No 117890.

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P20.016.C C-terminal truncation of NR2B subunits of NMDA receptor - functional characteristics of the GRIN2B nonsense mutation p.Glu839Ter

Roza Szlendak[1,2], *Nathalie Bouquier*[2], *Annie Varrault*[2], *Tristan Bouschet*[2], *Eymeline Pageot*[2], *Sylwia Rzońca-Niewczas*[1], *Dorota Hoffman-Zacharska*[1], *Julie Perroy*[2]

[1]*Department of Medical Genetics, Institute of Mother and Child, Warsaw, Poland,* [2]*Institute of Functional Genomics, IGF, University of Montpellier, CNRS, INSERM, Montpellier, France.*

Introduction: GRIN-related developmental-epileptic encephalopathies are rare genetic conditions caused by heterozygous mutations in Glutamate ionotropic receptors (GluNRs, NMDARs), ligand-gated ion channels with important roles in learning, memory and synaptic plasticity. NMDARs are heterotetramer and are composed of GluN1, GluN2 and GluN3 subunits. The C-terminal domains of subunits play an important role in their localization and synaptic function. We searched for "GRIN mutations" as targeted study. Research based on rare variants identified in patients is a powerful approach to study receptor function.

Materials and methods: We identified de novo heterozygous pathogenic mutation in *GRIN2B* gene - p.Glu839Ter. To study how this mutation alters receptor expression and biophysical properties we combined patch-clamp recordings, BRET experiments and immunocytochemistry, using HEK cells expressing wild type or mutated NMDA receptors subunits or patient fibroblasts reprogrammed into induced pluripotent stem cells (iPSCs) and differentiated into neurons.

Results: This mutation leads to truncation of the entire cytosolic C-tail of GluN2B subunits. The mutated GluN2B correctly interacts with wild type GluN2B and GluN1 subunits forming functional receptor. However, compared to wild type NMDAR, mutated one is less expressed at the cell surface and display a reduced NMDA current amplitude with higher sensitivity to magnesium blockade.

Conclusion: Ongoing experiments on patient-derived neurons might be a future platform for personal treatment of diseases with a broad spectrum of mutation. Functional analysis of the identified variants is an important step to interpret the clinical consequences of genetic variants and search for specific treatment for GRIN-related neuropathologies.

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P20.017.D Genome-wide DNA methylation analysis of the enteric nervous system in Hirschsprung disease

Jonathan D. Windster[1,2], *Musa Idris*[2,3], *Ruben G. Boers*[4], *Cornelius E. J. Sloots*[1], *René M. H. Wijnen*[1], *Veerle Melotte*[2,3], *Maria M. Alves*[2], *Robert M. W. Hofstra*[2]

[1]*Department of Pediatric Surgery, Erasmus University Medical Center - Sophia Children's Hospital, Rotterdam, Netherlands,* [2]*Department of Clinical Genetics, Erasmus University Medical Centre, Rotterdam, Netherlands,* [3]*Department of Pathology, GROW-School for Oncology*

and Developmental Biology, Maastricht University Medical Center, Maasstricht, Netherlands, [4]Department of Reproduction and Development, Erasmus University Medical Centre, Rotterdam, Netherlands.

Introduction: Hirschsprung disease (HSCR) is a neurocristopathy, characterized by an absence of enteric neurons in the distal part of the bowel. Epigenetic modifications, such as DNA methylation, are known to play crucial roles in the development of the enteric nervous system (ENS). However, the involvement of such modifications in HSCR pathogenesis, is still largely unknown.

Materials and methods: Colon tissue collected from HSCR patients (n =5) and controls (n = 5), was used to isolate ENS cells by enriching for the neuronal marker $p75^{NTR}$ with magnetically activated cell sorting. Cell-type specific genome-wide DNA methylation profiling (MeD-seq), was performed using the methylation-dependent restriction enzyme *Lpn*PI. Differentially methylated regions (DMRs) (>5.0-fold change) between HSCR and controls, were identified and used for a supervised hierarchical cluster analysis.

Results: The MeD-seq yielded 1541 DMRs in transcription starting sites (TSS), CpG islands and gene bodies. Gene ontology analysis of these DMRs showed enrichment (>2.0-fold change) of genes associated with regulatory pathways involved in nervous system development and differentiation. In the top 10 hypermethylated TSS in HSCR patients, we identified *MAB21L2*. Previous work from our group implicated this gene in ENS development by showing enteric aganglionosis in $mab21l2^{-/-}$ mutant zebrafish embryos.

Conclusions: Our data shows that DNA methylation is involved in HSCR pathogenesis, and suggests the involvement of *MAB21L2* in disease development. These findings are particularly interesting to further unravel new (modifier) genes involved in the etiology of HSCR, as well as to provide new potential markers for genetic counselling.

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P20.018.A Arm specific miRNAs expression analysis of hsa-miR-195 in non-small cell lung cancer

Veronika Y. Petkova[1], *Silva G. Kyurkchiyan*[1], *Darina Kachakova - Yordanova*[1], *Gergana S. Stancheva*[1], *Dora Marinova*[2], *Evgeni Mekov*[3], *Yanina Slavova-Marinova*[2], *Vanio Mitev*[1], *Radka Kaneva*[1]

[1]Molecular Medicine Center,Department of Medical Chemistry and Biochemistry, Medical Faculty, Sofia, Bulgaria, [2]University hospital "Medika", Ruse, Bulgaria, [3]Clinical Center for Lung Diseases, Medical Faculty, Medical University of Sofia, Sofia, Bulgaria.

Introduction: MicroRNAs (miRNAs) are small non-coding RNAs expressed in various tissues and cell types.They can help understanding the carcinogenesis of lung cancer and serve as potential diagnostic biomarkers for differentiating squamous cell carcinoma(SCC) and adenocarcinoma(ADC).The miR-195 hairpin gives rise to the "guide strand" miR-195-5p and the sister "passenger" strand miR-195-3p.This preference can be different and can change dynamically depending upon tissue types. The aim of the present study is to analyse and compare the expression patterns of miRNAs in ADC and SCC samples.

Methods: Fresh frozen tissue samples from 100 NSCLC patients (50ADC,50SCC) and adjacent normal tissues were examined.The expression of miRNAs was evaluated by qRT-PCR.The normalization of data, statistical and target prediction analyses were performed using R version 3.0.2 and GSEA with the Python package, GSEApy (version0.9.12).

Results: We assessed the expression levels of miR-195-5p and miR-195-3p between the ADC and adjacent normal tissues and found expression of miR-195-3p to be significantly differentially downregulated ($p < 0.001$). Expression of miR-195-5p was significantly downregulated ($p = 0.032$) between the SCC and adjacent normal. Gene Ontology (GO) analysis and pathway enrichment analysis was performed to investigate relationship between miRNA and targeted mRNA. Most affected signaling pathways were mTOR signaling pathway,Proteoglycans in cancer,DNA replication, Cell cycle, Wnt signaling pathway, FoxO signaling pathway.

Conclusions: The expression patterns of miRNAs and their target genes revealed both common and subtype specific signal pathways for ADC and SCC.Our results were in agreement with previous suggestions that miR-5p/-3p arms regulated signaling pathways critical to lung cancer development and chemoresistance.

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P20.019.B Hydralazine promotes the expression of pluripotency genes *OCT4* and *NANOG* in human somatic cells

Alain Aguirre-Vazquez[1,2], *Fabiola Castorena-Torres*[3], *Luis A Salazar-Olivo*[1], *Mario Bermúdez de León*[2]

[1]Instituto Potosino de Investigación Científica y Tecnológica, San Luis Potosí, Mexico, [2]Centro de Investigación Biomédica del Noreste, Instituto Mexicano del Seguro Social, Monterrey, Mexico, [3]Tecnologico de Monterrey, Escuela de Medicina y Ciencias de la Salud, Monterrey, Mexico.

Generation of human induced pluripotent stem cells (iPSC) has established promising opportunities for stem cell research, drug discovery and disease modeling. Despite their enormous potential in research, cell reprogramming is an inefficient process due to iPSCs contain epigenetic signatures from their cells of origin. This epigenetic memory constitutes one of the greatest obstacles in cell reprogramming. Currently, the search for small molecules that generate changes in the chromatin structure and reactivate the expression of genes related to cell reprogramming are of particular interest. Here we report the sole and combined effect of valproic acid (VPA), a class I selective HDAC inhibitor, and hydralazine (HYD), a DNA methyltransferase inhibitor, drugs over the expression of pluripotency genes in adult and newborn fibroblasts. Our results show that VPA upregulate *NANOG* expression by 2-fold in adult fibroblast. The combined effect of VPA and HYD nullifies the effect of each drug over pluripotency genes expression, except for *cMYC* which is increased in newborn fibroblasts. Interestingly, HYD significantly increase *OCT4* and *NANOG* expression by 2.5-fold and 4-fold, respectively in adult fibroblast. However, when HYD was added to enhance reprogramming efficiency no changes were observed. Currently, we are evaluating the molecular mechanisms by which HYD increases *OCT4* and *NANOG* expression. Preliminary results suggest that hypoxia-inducible factor 1-alpha, HIF1A, and the nuclear factor erythroid 2-related factor 2, NRF2, could be involved in the up-regulation of *OCT4* and *NANOG*. Finally, these data, for the first time, evidence that HYD regulates *OCT4* and *NANOG* expression in human somatic cells.

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P20.020.C Maternal-effect variants in *PADI6* and *NLRP2* genes associated with reproductive anomalies, Multilocus Imprinting Disorders (MLID) and Beckwith-Wiedemann syndrome (BWS)

Pierpaola Tannorella[1], *Luciano Calzari*[1], *Alessandro Vimercati*[1], *Ester Mainini*[1], *Davide Gentilini*[2,3], *Maria Teresa Bonati*[4], *Cecilia Daolio*[5], *Annalisa Pedrolli*[6], *Lidia Larizza*[1], *Silvia Russo*[1]

[1]*Istituto Auxologico Italiano, IRCCS, Cytogenetics and Molecular Genetics Laboratory, Milano, Italy,* [2]*Istituto Auxologico Italiano, IRCCS, Molecular Biology Laboratory, Unit of Bioinformatic and Statistical Genomic, Milano, Italy,* [3]*Department of Brain and Behavioral Sciences, University of Pavia, Pavia, Italy,* [4]*Istituto Auxologico Italiano, IRCCS, Service of Medical Genetics, Milano, Italy,* [5]*Pediatric Unit, Carlo Poma Hospital, Mantova, Italy,* [6]*IDivision of Pediatrics, S. Chiara Hospital, Outpatient Consultation for Rare Diseases, Trento, Italy*.

Deregulated methylation at single germline-DMRs is causative of Imprinting disorders (ID), diseases often characterized by growth alterations and/or by neurological symptoms. A heterogenous percentage of cases with a specific ID has been discovered to show deregulation in Multiple Loci (MLID). Here we refer to two patients with MLID and Beckwith-Wiedemann syndrome (BWS, OMIM # 130650), an overgrowth ID. The most frequent molecular BWS defect (50%) consists in the *KCNQ1OT1*:TSS-DMR hypomethylation and among these patients at least 30% of cases display MLID. The two mothers were investigated by WES disclosing novel pathogenic variants, respectively in *PADI6* and *NLRP2*. These genes are transcribed from the maternal genome, their mRNAs deposited in the oocyte, where they code for components of the subcortical maternal complex (SCMC), probably involved in the establishment of genomic imprinting and post-zygotic methylation maintenance. It is thought they play a role during the early stages of embryonic development. Two *PADI6* variants, p. Leu555Profs5ter and p.Asp547Asn, were detected in case 1: she had a reproductive history countersigned by nine miscarriages and a unique born child with BWS-MLID. The second mother showed a truncating homozygous variant in the *NLRP2* gene (p. Gln602ter). Conversely, this woman had an infertility history and underwent two ART attempts without success. Our findings enforce the concept that variants in SCMC genes may contribute to the birth of children with ID and MLID phenomenon; moreover, these variants may explain infertility and/or miscarriages often observed in these patients. Their study is extremely important in the context of family genetic counseling.

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P20.021.D Targeted deletion of a *cis* regulatory element of *abca4* using a paired guide RNA CRISPR Cas9 system in *Xenopus tropicalis*

Munevver Burcu Cicekdal[1,2], *Miriam Bauwens*[1], *Víctor López Soriano*[1], *Marjolein Carron*[1,2], *Elfride De Baere*[1], *Kris Vleminckx*[1,2]

[1]*Center for Medical Genetics and Department of Biomolecular Medicine (GE31), Ghent University and Ghent University Hospital, Ghent, Belgium,* [2]*Developmental Biology Unit, Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium.*

Introduction: *ABCA4*-retinopathy (including Stargardt disease, STGD1) is by far the most common single-gene inherited retinal disease (IRD). Non-coding variants in regions, such as *cis*-regulatory elements (CREs), may be implicated in missing heritability of *ABCA4*-associated disease. *Xenopus* (*X.*) *tropicalis* is an interesting model organism for IRD, having the major cell types of the human eye, and a true diploid genome. Here, we aimed to map and functionally study CREs of the *abca4* region and generate a stable knock-out of a CRE of *abca4* in *X. tropicalis* using CRISPR/Cas9 editing.

Material and Methods: Putative CREs of *abca4* were determined according to epigenetic markers H3K4me1 and Pol II in *X. tropicalis* whole embryo. Regulatory activity of putative CREs was tested using in vitro luciferase assays. Paired guide RNAs (gRNA) and Cas9 in *X. tropicalis* embryos was used to create a deletion of a selected CRE.

Results: A putative CRE of *abca4*, showing around 2-fold increase in luciferase activity compared to empty vector was selected as a target for disruption. Two gRNAs were designed as flanking the target CRE of *abca4*. The genomic region flanking the CRISPR target site was amplified and sequenced. Genome editing using a paired gRNA CRISPR/Cas9 system showed the deletion of the target CRE.

Conclusion: In conclusion, regulatory elements can be disrupted in model organisms using paired gRNAs. Regulatory animal models may contribute to the annotation of the non-coding genome and provide insights into the regulation of IRD genes such as *abca4*.Funding: EU ITN, grant No. 813490.

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P20.022.A biallelic PTRHD1 frameshift variant associated with intellectual disability

Ghalia Al-Kasbi, *Abeer Al-Saegh, Ahmed Al-Qassabi, Tariq Al Jabry, Fahad Al-Zadjali, Said Al-Yahyae, Almundher Al-Maawali*

Sultan Qaboos University, Muscat, Oman.

Background: PTRHD1 was recently proposed as the disease-causing gene in three families that shared the phenotype of intellectual disability and parkinsonism. Further reports and functional analysis to support the association are essential.

Objectives: To characterize the clinical phenotype and the molecular cause of the intellectual disability in four affected members of a consanguineous Arab family.

Methods: Clinical evaluation, neuroimaging studies, whole-exome sequencing, Sanger sequencing of candidate variants, reverse transcriptase PCR, Real-Time PCR, immunoblot and Isoelectric Focusing.

Result: A homozygous 28-nucleotide frameshift deletion introducing a premature stop codon in the PTRHD1 exon one was identified in the four affected family members. We further confirmed the apparent transcript escape of the nonsense-mediated mRNA decay pathway. Real-time PCR showed that mRNA expression of the mutant PTRHD1 is higher compared to wild-type. Western blotting identified a stable truncated mutant PTRHD1 protein expressed in the lymphocytes cells obtained from the peripheral blood of the patients. Isoelectric focusing proved that PTRHD1 protein in the affected individuals is truncated with MW of 8KDa with no significant expression difference relative to wild type protein.

Conclusion: We provide further evidence that PTRHD1 mutations are associated with autosomal-recessive childhood-onset intellectual disability and later symptoms of parkinsonism.

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P20.023.B Identification of long non-coding RNA controlling regulatory T cell identity

Véronique Adoue[1], *Samira Ghazali*[1], *Julie Noguerol*[1], *Manuel Lebeurrier*[2], *Karl Laviolette*[1], *Olivier Joffre*[1]

[1]*Infinity, Université Toulouse, CNRS, Inserm, UPS, Toulouse, France,* [2]*CHU de Rennes, Rennes, France, Rennes, France.*

Conventional CD4 T lymphocytes play a central role in the protection of the organism against a wide range of endogenous and exogenous dangers. Their action needs however to be tightly controlled by a population of regulatory T cells (Treg) endowed with immunosuppressive function. Treg critically control immune tolerance to self and prevent chronic inflammation. Long non-coding RNA (lncRNA) are acknowledged as important regulators of immune cell differentiation, but the repertoire of non-coding transcripts that control Treg development and function largely remains to be identified.

To achieve that goal, we fractionated the T cell compartment based on the nature, origin, activation status and location of the cells, and we analyzed the transcriptome of the 11 FACS-isolated subpopulations using a bioinformatic pipeline dedicated to lncRNA identification. In particular, since lncRNA are enriched in sequences derived from transposable elements, we combined specific tools and scripts in order to properly handle multi-mapped reads alignment.

This strategy allowed us to accurately annotate and to precisely estimate the expression level of 2316 new lncRNA specifically expressed in Treg and located nearby immune genes. Using conservation of synteny between mouse and human genomes, we next showed that many of these genes correspond to automatically annotated lncRNA genes in human, which overlap or are located nearby GWAS hits associated with immune-related disorders.

We are currently characterizing the function of the most promising candidates using in vitro and in vivo functional assays. Promising lncRNA could be considered as new therapeutic tool to control specific T cell immunity.

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P20.025.D Immunolocalization of epimarks: methylation (5mC) and hydroxymethylation (5hmC) in sperm DNA, and methylation (H3K4me3) and acetylation (H4K12ac) of lysine residues in histones, in differentially protaminated human spermatozoa

Marta Olszewska[1], *Oliwia Kordyl*[1], *Agnieszka Kralska*[1], *Marzena Kamieniczna*[1], *Piotr Jedrzejczak*[2], *Maciej Kurpisz*[1]

[1]*Institute of Human Genetics, Polish Academy of Sciences, 60-479, Poland,* [2]*Department of Infertility and Reproductive Endocrinology, Poznan University of Medical Sciences, 60-535, Poland.*

Introduction: A special role in etiology of male infertility play epigenetic modifications of DNA and histones, including methylation (5mC), hydroxymethylation (5hmC), histones' lysine residues methylation or acetylation. Also, the link between sperm quality and the chromatin protamination status is known. Our aim was to determine the relationship between 5mC, 5hmC, H3K4me3 and H4K12ac and the sperm chromatin protamination, in three sperm subpopulations: properly-protaminated, less-protaminated, and deprotaminated.

Materials and Methods: Spermatozoa from 31 patients with oligo-/oligoasthenozoospermia and 28 normozoospermic controls were evaluated using a sequential staining protocol (for the first time), which allowed to analyze the epimarks' level and their nuclear localization by immunofluorescent stainings on the same spermatozoon with determined chromatin protamination status (aniline blue staining).

Results: The protamination levels of sperm chromatin and 5mC/5hmC were decreased in the infertile patients, followed by increased values of H3K4me3 and H4K12ac, and higher inter-individual heterogeneity of all epimarks. All epimarks levels were highest in properly-protaminated spermatozoa (both groups). H4K12ac localization was shifted towards sperm acrosome in majority of patients. A relationship between 5mC/5hmC and sperm motility or morphology was identified.

Conclusions: The high DNA 5mC and low H3K4me3 levels are markers for properly-protaminated spermatozoa, documenting the correct spermatogenesis. Its disruption may indicate a reproductive failure revealed as decreased quality of seminological parameters and/or fertility problems. Disturbances in both histones' epimarks observed in this study may influence fertilization process due to abnormal chromatin compaction in acrosomal area and then aberrant gene transcription in early embryo development. **Funding:** 2015/17/D/NZ5/03442, National Science Centre in Poland

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P20.026.A Interaction between asbestos exposure and stochastic epigenetic mutations in malignant pleural mesothelioma

Giovanni Cugliari[1], *Alessandra Allione*[1], *Simonetta Guarrera*[2,3], *Elisabetta Casalone*[1], *Davide Gentilini*[4], *Federica Grosso*[5], *Daniela Ferrante*[6,7], *Marika Sculco*[8], *Anna Aspesi*[8], *Roberta Libener*[9], *Enrica Migliore*[10,11,12], *Dario Mirabelli*[10,11,12], *Corrado Magnani*[6,7,10], *Irma Dianzani*[8,10], *Giuseppe Matullo*[1,10,13]

[1]*Department of Medical Sciences, University of Turin, Turin, Italy,* [2]*Italian Institute for Genomic Medicine, IIGM, Candiolo, Italy,* [3]*Candiolo Cancer Institute, FPO - IRCCS, Candiolo, Italy,* [4]*Department of Brain and Behavioral Sciences, University of Pavia, Pavia, Italy,* [5]*Mesothelioma Unit, Azienda Ospedaliera SS Antonio e Biagio e Cesare Arrigo, Alessandria, Italy,* [6]*Unit of Medical Statistics, Department of Translational Medicine, University of Piemonte Orientale, Novara, Italy,* [7]*Cancer Epidemiology Unit, CPO-Piemonte, Novara, Italy,* [8]*Department of Health Sciences, University of Piemonte Orientale, Novara, Italy,* [9]*Department of Integrated Activities Research and Innovation - Azienda Ospedaliera SS. Antonio e Biagio e Cesare Arrigo, Alessandria, Italy,* [10]*Cancer Epidemiology Unit, Department of Medical Sciences, University of Turin, Turin, Italy,* [11]*Cancer Epidemiology Unit, CPO Piemonte, Turin, Italy,* [12]*Inter-departmental Center for Studies on Asbestos and Other Toxic Particulates "G. Scansetti", University of Turin, Turin, Italy,* [13]*Medical Genetics Unit, AOU Città della Salute e della Scienza, Turin, Italy.*

BACKGROUND: Malignant pleural mesothelioma (MPM) is a rare and aggressive neoplasm strongly associated with asbestos exposure. The aim of this study is to investigate the relationship between stochastic epigenetic mutations (SEMs) and MPM with the aim to better characterize the burden between MPM cases and controls. Interaction between asbestos exposure and SEM was evaluated to infer on the MPM odds ratio (OR).

Methods: We analyzed methylation levels through the Human-Methylation450 Beadchip in a population of 300 (163 cases and 137 controls) subjects. Multivariate regression analysis considering

age, gender, population stratification and WBCs composition was performed. As second outcome, we investigated the effect of asbestos exposure in cases and controls. Lastly, interaction analysis was performed to better characterize the MPM OR.

Results: We demonstrated that mean of the number of total SEMs (hypo and hyper) was higher in cases respect to controls. In particular, hypo-SEMs showed a mean difference between cases and controls about three-fold higher than hyper-SEMs. Moreover, mean SEMs increases in relation to asbestos exposure in cases but not in exposed controls. Considering asbestos exposure and SEM statistically significant interaction effect was found considering categorical clustering by medians.

Conclusions: The SEMs approach can add information at the level of epigenetic evaluation in the context of MPM. SEMs can be used as outcome or mediator in association models in order to better understand its contribution to MPM development. Considering SEM occurrence and asbestos exposure levels may allow clinicians to better evaluate MPM risk.

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P20.027.B Directly detect and phase genomic 5mC methylation with high reproducibility and low bias using Nanopore sequencing

***Rocío Esteban**, Irina-Alexandra Vasilescu, Marcus H. Stoiber, Daniel J. Turner, David Stoddart, Eoghan Harrington, Sissel Juul, Philipp Rescheneder*

Oxford Nanopore Technologies Ltd, Oxford, United Kingdom.

Epigenetics, the study of heritable phenotypic changes that do not involve alteration of the nucleotide sequence, plays a key role in gene expression and has been associated with many diseases. As PCR removes base modifications, their detection via traditional sequencing technologies requires the use of special library preparation steps to convert nucleotides according to their methylation status. These additional steps are labour intensive, damage DNA, introduce biases and complicate analysis.With nanopore sequencing, amplification and other sample prep is not required, enabling DNA and RNA modifications to be preserved and directly sequenced and detected. Nanopore sequencing therefore greatly simplifies whole genome methylation calling.Here we benchmarked the performance of nanopore 5mC methylation calling to assess whether it could be used as a replacement for traditional methods of 5mC detection. We compared nanopore methylation calls with publicly available bisulphite sequencing datasets of the NA12878 cell line. We showed the effect of sequencing biases like GC bias and uniformity of coverage and assed how much of the human genome can be called by both technologies at different coverage levels. We found that at 20X Nanopore outperforms bisulphite sequencing datasets with more than twice the sequencing depth. Furthermore, we found high correlation (>0.9) of methylation frequencies compared to bisulphite and high reproducibility between runs on a per base level as well as for larger features like CpG islands. Finally, we successfully demonstrated how to combine methylation calling with read phasing to identify 5mC methylation status independently for both haplotypes and identify imprinted genes in humans.

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P20.028.C Characteristics of DNA methylation of the regulatory region of the *MLH1* gene in peripheral blood leukocytes of patients with common age-related diseases

***Nadezhda P. Babushkina**, Anton V. Markov, Irina A. Goncharova, Ramil R. Salahov, Iuliia A. Koroleva, Elena Yu Bragina, Aleksei A. Zarubin, Alexei Sleptcov, Aksana N. Kucher, Maria S. Nazarenko M.S*

Research Institute of Medical Genetics, Tomsk National Research Medical Center, Tomsk, Russian Federation.

MLH1 protein is one component of a system of DNA mismatch repair. These proteins serve crucial functions in many biological processes. MLH1 epimutations is a cause of Lynch syndrome. However, there is not data about the DNA methylation of the regulatory region of the MLH1 gene in leukocytes of patients with common age-related diseases. We studied the DNA methylation level of the MLH1 promoter region in peripheral blood leukocytes of patients with severe carotid atherosclerosis ($n = 22$), Huntington's disease ($n = 14$), lung cancer ($n = 8$) and healthy individuals ($n = 27$) by bisulfite NGS on the Illumina platform. There was no statistically significant difference of DNA methylation level of the MLH1 promoter region (GRCh37/hg19; chr3:37,033,249-37,033,762) between groups of patients and healthy individuals, although the mean methylation levels for individual CpG-sites varied significantly (from 0.1 to 12%). We found that lung cancer patients differ significantly in the level of methylation of the CpG-site chr3: 37,033,373 from healthy people and patients with Huntington's disease ($p = 0.046$ and $p = 0.0196$, respectively). The highest average methylation level for most CpG-sites was detected in leukocytes of patients with lung cancer (34.7%-73,0%). Patients with Huntington's disease had the lowest average methyl tion level for most CpG-sites in leukocytes (26.5%-65.0%).These data indicate the differences in mean methylation levels of individual CpG-sites in Huntington's disease and lung cancer but without a total change in the methylation status of studied region of the MLH1 gene. This work was carried out with partial support of the RFBR grant No. 19-015-00391-A

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P20.029.D New diagnostic tool for multi-locus imprinting disturbances

***Eguzkine Ochoa**[1], Sunwoo Lee[1], Benoit Lan-Leung[1], Renuka Dias[2,3,4], Ken K. Ong[5,6], Jessica A. Radley[7,8], Gustavo Pérez de Nanclares[9], Rosa Martinez[9], Graeme Clark[10], Ezequiel Martin[10], Luis Castaño[9], Leonardo Bottolo[11,12,1], Eamonn R. Maher[1]*

[1]Department of Medical Genetics, Cambridge, United Kingdom, [2]Institutes of Metabolism and Systems Research, Birmingham, United Kingdom, [3]Centre for Endocrinology, Diabetes and Metabolism, Birmingham, United Kingdom, [4]Department of Paediatric Endocrinology and Diabetes, Birmingham Children's Hospital NHS Foundation Trust, Birmingham, United Kingdom, [5]MRC Epidemiology Unit,

Institute of Metabolic Science, University of Cambridge School of Clinical Medicine, Cambridge, United Kingdom, [6]Department of Paediatrics, University of Cambridge School of Clinical Medicine, Cambridge Biomedical Campus, Cambridge, United Kingdom, [7]West Midlands Regional Clinical Genetics Service and Birmingham Health Partners, Birmingham Women's and Children's Hospitals NHS Foundation Trust, Birmingham, United Kingdom, [8]London North West Regional Genetics Service, St. Mark's and Northwick Park hospitals, Harrow, United Kingdom, [9]Biocruces Bizkaia Health Research Institute, Hospital Universitario Cruces, CIBERDEM, CIBERER, Endo-ERN, University of the Basque Country (UPV-EHU), Barakaldo, Spain, [10]Stratified Medicine Core Laboratory NGS Hub, Cambridge Biomedical Campus, Cambridge, United Kingdom, [11]The Alan Turing Institute, London, United Kingdom, [12]MRC Biostatistics Unit, University of Cambridge, Cambridge, United Kingdom.

Multi-locus imprinting disturbance (MLID) is defined as multiple imprinting defects across the genome and has been described in miscarriages, recurrent hydatidiform moles and in some congenital imprinting disorders (CIDs). These imprinting defects are produced by epimutations but their underlying cause in most cases is unknown. An increased risk of MLID has been associated with assisted reproductive technology births and genetic causes may be identified in a small subset of patients (e.g. biallelic *ZFP57* mutations). The specific impact of MLIDs on clinical phenotype is not well defined though some patients will have discordant epigenotype-phenotype. We analysed 145 individuals with a CID and 70 healthy controls with ImprintSeq, a custom targeted methylation sequencing panel capable to interrogate 63 imprinting differentially methylated regions (iDMRs). We defined 3 standard deviation confidence interval of mean methylation level (MML) per iDMR in the healthy controls and we distinguished loss-of-methylation (LOM) and gain-of-methylation (GOM) when MML per iDMR is below or above this interval. We classified the significant signals detected in high (HMA) and moderate methylation alteration (MMA) based on the difference with MML in controls. Using proposed diagnostic criteria for MLID (in addition to the primary CID-associated diagnostic epimutation) of either a LOM/GOM HMA at a CID-associated iDMR or LOM/GOM HMAs at two non-CID iDMRs, the frequency of MLID in individual CIDs varied between 0% to 42%. Profiling larger cohorts of CID patients will contribute to determine the significance of HMAs at specific iDMRs for patient phenotype.*This research was supported by the Cambridge NIHR BRC*

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P20.030.A Characterization of the functional enhancers in human neural stem cells

***Elena Perenthaler**, Soheil Yousefi, Ruizhi Deng, Anita Nikoncuk, Tahsin Stefan S. Barakat*

Erasmus Medical Center, Rotterdam, Netherlands.

The development of the cerebral cortex is a complex and dynamic process. Alterations at any stage can result in a wide range of neurodevelopmental disorders (NDDs), that are a common cause of developmental delay, intellectual disability, and epilepsy. Exome sequencing greatly increased the diagnostic yield of genetic forms of NDDs, allowing the identification of variations in hundreds of genes. Nevertheless, many cases remain genetically unexplained, hinting at variations in the non-coding genome. Among these non-coding regions are the understudied enhancers, *cis*-acting elements that control gene-expression in a temporal and tissue-specific manner during many key-developmental processes. Here, we combined analysis of transcription factor binding sites, histone modifications (ChIPseq) and open chromatin regions (ATACseq) with the massively parallel reporter assay ChIP-STARR-seq to identify the subset of functional enhancers in human neural stem cells, an in vitro model reflecting early brain development. This led to a genome-wide, quantitative map of enhancer activity of relevance for neurodevelopmental disorders.

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P20.031.B Hybrid minigene assay: an efficient tool to characterize mRNA splicing profiles of *NF1* variants

***Valeria Morbidoni**[1,2], Elisa Baschiera[1,2], Monica Forzan[2], Valentina Fumini[2], Dario Seif Ali[2], Gianpietro Giorgi[2], Lisa Buson[1,2], Maria Andrea Desbats[1,2], Matteo Cassina[2], Maurizio Clementi[2], Leonardo Salviati[1,2], Eva Trevisson[1,2]*

[1]Istituto di Ricerca Pediatrica - Fondazione Città della Speranza, Padova, Italy, [2]Clinical Genetics Unit, Department of Women's and Children's Health, University of Padova, Padova, Italy.

Introduction: Neurofibromatosis 1 (NF1) is caused by heterozygous loss of function mutations in *NF1*. Although patients are diagnosed according to clinical criteria and few genotype-phenotype correlations are known, molecular analysis remains important. *NF1* displays allelic heterogeneity, with a high proportion of variants acting on splicing, including deep intronic alleles and changes outside the canonical splice sites, making validation problematic. NGS technologies integrated with MLPA have largely overcome RNA-based techniques that are faster and with high yield, but do not detect splicing defects.

Materials and Methods: We set up and employed a rapid minigene-based system to test the effect(s) of 29 intronic and exonic variants in *NF1*, which were identified in patients during molecular analyses, on splicing.

Results: The minigene assay allowed to assess the effect(s) on splicing for all the variants we examined and showed the coexistence of multiple mechanisms of splicing alterations for seven of them. In one *de novo* substitution identified in a sporadic patient with a mild phenotype, a leaky effect on splicing was documented suggesting a new genotype-phenotype correlation.

Conclusions: Our splicing assay proved to be a reliable and fast method to validate novel *NF1* variants potentially affecting splicing and to detect hypomorphic effects that might underline milder phenotype, avoiding the requirement of patient's RNA. Funding: Grants from Italian Ministry of Health (Young Researcher, Grant number GR-2016-02362779) and Istituto di Ricerca Pediatrica Fondazione Città della Speranza IRP (Grant number 19/10).

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P20.033.D Identification of the regulatory network at the nsCL/P associated GWAS locus 1p36.13

***Ronja Hollstein**[1], Frederic Thieme[1], Julia Welzenbach[1], Hanna K. Zieger[1], Julia Schröder[1], Magdalena Laugsch[2], Kerstin U. Ludwig[1]*

[1]Institute of Human Genetics, University of Bonn, School of Medicine & University Hospital Bonn, Bonn, Germany, Bonn, Germany, [2]Institute of Human Genetics, University of Heidelberg, Heidelberg, Germany, Heidelberg, Germany.

Non syndromic cleft lip with/without cleft palate (nsCL/P) is among the most common birth defects. The condition has a multifactorial etiology with a strong genetic component. Within the last decade, more than 40 risk loci have been identified through GWAS and the majority of risk loci map to non-coding regions. The development of nsCL/P takes place during the first 4-10 weeks of embryogenesis, and is presumed to involve several transient cell systems. Thus, the translation of disease-associated non-coding variants into a functional model is challenging and further limited by the availability of cellular models and the presumed time point- and tissue specific effect of the variants. Our group has previously shown that associated risk variants for nsCL/P are strongly enriched in active regulatory elements of human neural crest cells (hNCCs). Preliminary 4C data using the sentinel SNV of the newly identified risk locus 1p36.13 as viewpoint suggest an interaction with *GRHL3*, a known cleft palate-associated gene. Further, we used an *in silico* approach to screen the sentinel SNV for possible transcription factor binding sites. Interestingly, we identified binding motifs of key regulators of neural crest development, such as TFAP2A and SNAI2, to be affected by the SNV. To unravel the underlying regulatory network, we are establishing a combined approach of circular chromosome conformation capture (4C) and ChIP-seq in iPSC-derived hNCCs, and first results will be presented at the conference. Overall, this project will contribute to the identification and understanding of regulatory networks involved in the development of nsCL/P.

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P20.035.B From man to mouse: The discovery and validation of CYR61 as a regulator of body composition

Sophia Metz, *Katja Thorøe Michler, Matthew Paul Gillum, Tuomas Oskari Kilpeläinen*

Novo Nordisk Foundation Center for Basic Metabolic Research, Copenhagen, Denmark.

Obesity is a major contributor to the global burden of chronic disease. There is currently a great unmet need for developing effective and safe anti-obesity treatments. Genomic studies provide novel molecular targets for obesity treatment by discovering genes and pathways involved in obesity. We have recently identified an association between the rare variant Ser316Cys in *CYR61*, implicated in angiogenesis, and increased body fat percentage ($P = 1.1x10^{-9}$), particularly trunk fat percentage ($3.8x10^{-11}$). The Cys316 minor allele was associated with a 0.4 % higher body fat percentage and 0.5% higher trunk fat percentage. In a mouse model overexpressing human-Cyr61 (hCyr61) in the fat tissue, we see an increased body fat percentage under a high fat diet (HFD), due to a switch in body composition (higher fat mass, lower lean mass), while body weight did not differ from WT mice. The mean area of adipocytes was increased in the hCyr61 overexpressing mice ($p = 0.009$), reflecting increased adipocyte hypertrophy. The endogenous Cyr61 remained unaffected by hCyr61, but was abundantly expressed in all fat depots, supporting a hypothesis of *CYR61* being involved in fat tissue development. Our results suggest a critical role of *CYR61* in the regulation of body composition, possibly mediated by angiogenesis and adipocyte growth. Ongoing in vitro experiments will provide further insights into the mechanistic role of *CYR61* in the development of obesity and metabolic dysfunction. Ultimately, understanding the mechanisms of how *CYR61* regulates body composition may enable new therapeutic options for the treatment of obesity by lowering fat mass and retaining lean mass.

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P20.036.C Genome-wide DNA methylation analysis of a cohort of 41 patients affected by Oculo-Auriculo-Vertebral Spectrumpatients affected by Oculo-Auriculo-Vertebral Spectrum (OAVS)

Valentina Guida[1], *Luciano Calzari*[2], *Maria Teresa Fadda*[3], *Francesca Piceci-Sparascio*[1,4], *Maria Cristina Digilio*[5], *Laura Bernardini*[1], *Francesco Brancati*[6,7], *Teresa Mattina*[8], *Daniela Melis*[9], *Francesca Forzano*[10], *Silvana Briuglia*[11], *Tommaso Mazza*[12], *Sebastiano Bianca*[13], *Enza Maria Valente*[14,15], *Leila Bagherjad Salehi*[16], *Paolo Prontera*[17], *Pagnoni Mario*[3], *Romano Tenconi*[18], *Bruno Dallapiccola*[5], *Giorgio Iannetti*[3], *Luigi Corsaro*[19], *Alessandro De Luca*[1], *Davide Gentilini*[2,19]

[1]*Medical Genetics Division, Fondazione IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy,* [2]*Istituto Auxologico Italiano IRCCS, Bioinformatics and Statistical Genomics Unit, Cusano Milanino, Italy,* [3]*Department of Maxillofacial Surgery, Sapienza University of Rome, Rome, Italy,* [4]*Department of Experimental Medicine, Sapienza University of Rome, Rome, Italy,* [5]*Genetics and Rare Diseases Research Division, Ospedale Pediatrico Bambino Gesù, IRCCS, Rome, Italy,* [6]*Department of Life, Health and Environmental Sciences, Unit of Medical Genetics University of L'Aquila, L'Aquila, Italy,* [7]*IRCCS San Raffaele Pisana, Rome, Italy,* [8]*Medical Genetics, Department of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy,* [9]*Department of Medicine, Surgery and Dentistry, University of Salerno, Salerno, Italy,* [10]*Clinical Genetics Department, Guy's & St Thomas' NHS Foundation Trust, London, United Kingdom,* [11]*Medical Genetics, University of Messina, Messina, Italy,* [12]*Unit of Bioinformatics, Fondazione IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy,* [13]*Centro di Consulenza Genetica e Teratologia della Riproduzione, Dipartimento Materno Infantile, ARNAS Garibaldi Nesima, Catania, Italy,* [14]*Department of Molecular Medicine, University of Pavia, Pavia, Italy,* [15]*IRCCS Mondino Foundation, Pavia, Italy,* [16]*Tor Vergata University Hospital, Medical Genetics Unit, PTV, Rome, Italy,* [17]*Medical Genetics Unit, University of Perugia Hospital SM della Misericordia, Perugia, Italy,* [18]*Department of Pediatrics, Clinical Genetics, Università di Padova, Padova, Italy,* [19]*Department of Brain and Behavioral Sciences, University of Pavia, Pavia, Italy.*

Introduction: Oculo-auriculo-vertebral-spectrum (OAVS) is a rare disorder of craniofacial morphogenesis involving the first and second branchial arch derivatives. The clinical phenotype is extremely heterogeneous with ear anomalies, hemifacial microsomia, ocular defects, and vertebral malformations being the main features. Chromosomal anomalies as well as point mutations have been documented in some OAVS patients, but the etiology of the disease remains largely unknown. A multifactorial origin has been proposed, including the involvement of environmental/epigenetic mechanisms. To search for the epigenetic mechanisms contributing to disease, we explored the DNA-methylation profile of OAVS individuals.

Material and methods: study-cohort included 41 OAVS-affected subjects and the tissue-matched methylation profiles of 48 anonymous healthy individuals. DNA-methylation profiles were obtained by using the Illumina Infinium Methylation 450K Beadchip.

Results: analysis was first carried out comparing OAVS patients with controls at the group level. This approach revealed a moderate epigenetic variation in a large number of genes implicated in basic chromatin dynamics such as DNA packaging and protein-DNA organization. An alternative approach based on

the analysis of individual profiles to search for Stochastic Epigenetic Variants (SEVs) identified an increased number of SEVs in OAVS subjects compared to controls. Although no recurrent deregulated enriched regions were found, isolated patients harboring suggestive epigenetic deregulations were identified.

Conclusions: The recognition of a different DNA methylation pattern in the OAVS cohort and the identification of isolated patients with suggestive epigenetic variations provide consistent evidence for the contribution of epigenetic mechanisms to the etiology of this complex and heterogeneous disorder. Funding: IMH RC2019, RC2020

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P20.037.D Weighted gene co-expression network analysis identifies critical altered pathways and hub genes in high-grade serous ovarian cancer

Magdalena Niemira[1], *Karolina Chwialkowska*[2], *Agnieszka Bielska*[1], *Anna Szalkowska*[1], *Iwona Sidorkiewicz*[1], *Urszula Korotko*[2], *Anna Erol*[1], *Justyna Raczkowska*[1], *Gabriela Sokolowska*[1], *Katarzyna Doroszko*[1], *Sylwia Chludzinska*[3], *Jacek Szamatowicz*[4], *Pawel Knapp*[5], *Miroslaw Kwasniewski*[2], *Joanna Reszec*[3], *Marcin Moniuszko*[6], *Adam Kretowski*[1,7]

[1]*Clinical Research Centre, Medical University of Bialystok, Bialystok, Poland,* [2]*Centre for Bioinformatics and Data Analysis, Medical University of Bialystok, Bialystok, Poland,* [3]*Department of Medical Pathomorphology, Medical University of Bialystok, Bialystok, Poland,* [4]*Department of Gynecology and Gynecological Oncology, Medical University of Bialystok, Bialystok, Poland,* [5]*University Oncology Center, Medical University of Bialystok, Bialystok, Poland,* [6]*Department of Regenerative Medicine and Immune Regulation, Medical University of Bialystok, Bialystok, Poland,* [7]*Department of Endocrinology, Diabetology and Internal Medicine, Medical University of Bialystok, Bialystok, Poland.*

Introduction: High-grade serous ovarian cancer (HGSOC) is the most common histological subtype of epithelial ovarian cancer, with a five-year survival rate below 30%. While the disease limited only to the ovaries can be cured in up to 90% of patients, most cases are diagnosed at a late stage. Therefore, a more effective understanding of the importance of biological pathways and the relationship between major genes in HGSOC in the perspective of searching for new targets are still urgently required.

Materials and methods: The transcriptional changes between tumour (n = 33) and normal (n = 33) ovary tissues were investigated by RNA-seq. Gene ontology (GO), canonical pathways analysis (IPA), gene set enrichment analysis (GSEA) and weighted gene co-expression network analysis (WGCNA) to identify co-expressed modules and hub genes were used to explore the biological functions of the dysregulated genes.

Results: The analysis revealed that 2718 deregulated genes were related to developmental process, cell cycle, cytokines, inflammatory response and apoptosis. By using WGCNA was revealed 15 co-expression modules and identified the driving module for HGSOC associated with the tumour size, the presence of lymph node metastasis and the distant metastasis. The hub genes including *FOXK2*, *EFNA5*, *XPC*, *VGLL4*, *ELP1*, *MKKS*, *LIN7B*, *STS*, *ZNF23* and *ZNF71*, which may be potential biomarkers or therapeutic targets for HGSOC, have been identified.

Conclusions: The findings of the present study could improve our understanding of the molecular pathogenesis of HGSOC and shed light on further investigation. *Data were generated by the Centre for Artificial Intelligence of the Medical University of Bialystok.*

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P20.038.A Mitochondrial D-loop region methylation and copy number are not altered in peripheral blood of Parkinson's disease patients

Andrea Stoccoro[1], *Adam R. Smith*[2], *Claudia Del Gamba*[3], *Katie Lunnon*[2], *Filippo Baldacci*[3], *Lucia Migliore*[1], *Fabio Coppedè*[1]

[1]*Department of Translational Research and of New Surgical and Medical Technologies, University of Pisa, Pisa, Italy,* [2]*University of Exeter Medical School, Exeter, United Kingdom,* [3]*Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy.*

Introduction: In recent years growing evidence on a potential role of altered mitochondrial DNA methylation in several diseases has emerged, although until now little attention has been given to neurodegenerative diseases. Recently, we reported that methylation levels of the mitochondrial displacement loop (D-loop) region, which regulate mitochondrial DNA (mtDNA) replication, are impaired in peripheral blood cells of late-onset Alzheimer's disease and amyotrophic lateral sclerosis patients. The aim of the current research was to investigate D-loop methylation levels and mtDNA copy number in Parkinson's disease (PD) patients.

Materials and Methods: Blood samples have been collected from 30 PD and 30 age and sex matched control subjects. DNA methylation analyses have been performed by means of Methylation Sensitive High Resolution Melting (MS-HRM) and pyrosequencing techniques, while mtDNA copy number by means of quantitative PCR.

Results: MS-HRM and pyrosequencing analyses provided very similar D-loop methylation levels in PD patients and control subjects, and no differences between the two groups have been observed. Moreover, use of L-dopa and duration of the disease had no effect on D-loop methylation levels in PD patients. Also mtDNA copy number did not differ between PD patients and control subjects. A significant inverse correlation between pyrosequencing D-loop methylation levels and age at sampling of the individuals enrolled has been detected ($r = -0.53$, $p < 0.0001$).

Conclusions: Current results suggest that D-loop methylation levels are not altered in peripheral blood of PD patients and reinforce previous evidence that peripheral blood mtDNA methylation are sensitive to ageing.

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P20.039.B Transcriptome analysis of the effects of melanocortin and tuftsin analogues in rat brain

Ivan B. Filippenkov[1], *Vasily V. Stavchansky*[1], *Natalya Yu Glazova*[1], *Natalia G. Levitskaya*[2], *Elena A. Sebentsova*[1], *Daria D. Khukhareva*[2], *Liya V. Valieva*[1], *Anna E. Golubovich*[3], *Nikolai F. Myasoedov*[1], *Svetlana A. Limborska*[1], *Lyudmila V. Dergunova*[1]

[1]Institute of Molecular Genetics of National Research Centre «Kurchatov Institute», Moscow, Russian Federation, [2]Lomonosov Moscow State University, Moscow, Russian Federation, [3]I.M. Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russian Federation.

Introduction: Synthetic peptides have a wide range of clinical effects. Of particular interest, peptides based on adrenocorticotropic hormone (ACTH) and tuftsin are used as drugs to prevent the effects of cerebral ischemia and stress. However, their precise mechanisms of action within the body remain unclear to date. Here, we used high-throughput RNA sequencing (RNA-Seq) to analyze differential expressed genes (DEGs) in the frontal cortex of rats produced by melanocortin (ACTH(4–7)PGP (Semax), ACTH (6–9)PGP), and tuftsin (Selank) analogues under normal physiological conditions.

Materials and Methods: Wistar rats, RNA-Seq, real-time RT-PCR, bioinformatics.

Results: Using RNA-Seq we revealed 257, 100, and 228 DEGs with cut-off>1.5 and padj < 0.05 at 22.5h after the first administration of Semax, Selank, and ACTH(6–9)PGP, respectively. Moreover, all the peptides tested had a strong effect on the expression of genes (e.g., RT1-Ba, Cxcl13, RT1-Db1, RT1-Da) associated with the immune system. Simultaneously, each of the peptides had a specific effect on the transcriptome. We revealed DEGs of nucleic acids and protein metabolism (Tlr7, Cd48, Stk17b, Eif2ak2, Fli1) for Selank; DEGs linked to lipid binding (Apol3, Apol9a), and the regulation of ion channels (Grin2a, Arc, Slc6a13) for Semax; and DEGs associated with the functioning of proteasomes (Psma8, Psmb11, Psmb8, Psmb9), and DNA replication (Mcm3) for ACTH(6–9)PGP action.

Conclusion: Our data suggest that when studying the effects of regulatory peptides on the transcriptome under pathological conditions, it is necessary to consider their effects under normal physiological conditions. This work was supported by grant from the Russian Science Foundation 19-14-00268.

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P20.040.C A common variant in the *PHOX2B* 3'UTR is associated with infant life-threatening and sudden death events in the Italian population

*Tiziana Bachetti[1,2], Simona Bagnasco[1], Raffaele Piumelli[3], Antonella Palmieri[4], **Isabella Ceccherini**[1]*

[1]Laboratorio di Genetica e Genomica delle malattie rare, IRCCS Giannina Gaslini, Genova, Italy, [2]University of Genova-DISTAV, Genova, Italy, [3]Centro per i Disturbi Respiratori nel Sonno-Centro Regionale SIDS, Firenze, Italy, [4]Dipartimento di Emergenza, Centro SIDS-ALTE, Genova, Italy.

Heterozygous mutations in the Paired like homeobox 2b (*PHOX2B*) gene are responsible for congenital central hypoventilation syndrome (CCHS), a rare autosomic dominant monogenic disease caused by a compromised development of the autonomic nervous system (ANS).

CCHS is characterized by a predominant respiratory phenotype due to sudden hypoxic manifestation, a condition resembling two other unexplained perinatal disorders caused by defective ANS, apparent life-threatening event (ALTE) and Sudden and Unexpected Infant Death (SUID), among which the vast majority is represented by Sudden Infant Death Syndrome (SIDS).

However, while CCHS is a Mendelian disorder, ALTE and SIDS are complex traits, where common genetic variants, together with external factors, may exert an additive effect with symptoms likely manifesting only over a "threshold".

Given the similarities observed among the three above mentioned perinatal disorders, in order to search a genetic role of *PHOX2B* in both complex traits we have analysed the frequency of *PHOX2B* common variants in two groups of Italian idiopathic ALTE (IALTE) and SUIDs/SIDS patients.

We have found that the c*161G>A (rs114290493) SNP of the 3'UTR of *PHOX2B* resulted overrepresented in the two sets of patients compared to population matched healthy controls; moreover, it was associated with a decreased activity of the PHOX2B 3'UTR mediated by miR-204, likely resulting in a reduced *PHOX2B* expression, in accordance with observations made in specimens derived from SIDS patients.

Overall these results suggest that c*161G>A causes a loss-of-function effect of *PHOX2B*, and can be considered a susceptibility factor in Italian sudden unexplained perinatal life-threatening or fatal disorders

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P20.041.D Extensive placental methylation profiling in normal pregnancies

***Ornella Rondinone**[1], Alessio Murgia[1], Jole Costanza[1], Silvia Tabano[2,3], Margherita Camanni[1], Luigi Corsaro[4], Laura Fontana[1,5], Patrizia Colapietro[2], Luciano Calzari[6], Silvia Motta[1], Carlo Santaniello[1], Tatjana Radaelli[7], Davide Gentilini[4,6], Silvia Maria Sirchia[5], Monica Miozzo[1,5]*

[1]Research Laboratories Coordination Unit, Fondazione IRCCS Ospedale Ca' Granda Policlinico Milano, Milan, Italy, [2]Department of Pathophysiology and Transplantation, Medical Genetics, Università degli Studi di Milano, Milan, Italy, [3]Laboratory of Medical Genetics, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, [4]Department of Brain and Behavioral Sciences, Università di Pavia, Pavia, Italy, [5]Medical Genetics, Department of Health Sciences, Università degli Studi di Milano, Milan, Italy, [6]Bioinformatics and Statistical Genomics Unit, Istituto Auxologico Italiano IRCCS, Cusano Milanino, Milan, Italy, [7]Department of Obstetrics and Gynecology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy.

The placenta is the transient organ of primary importance during pregnancy, intimately connecting mother and fetus and ensuring nutrients and oxygen to the embryo, waste disposal and production of hormones. In order to allow the pregnancy to progress, even in presence of unfavorable conditions (of maternal or fetal origin), the placenta can adapt dynamically, a process favored by epigenetic modifications. These modifications may persist after birth, as an "epigenetic memory", and influence post-natal health (DoHad hypothesis by D. Barker). In order to define the placenta methylome compared to cord blood by means of an ontology- driven approach, we explored the LINE-1 methylation profile in cord blood and placenta samples from 154 uncomplicated full-term pregnancies, and the genome-wide methylation pattern by methylation array (Infinium EPIC array) in ten pregnancies and by targeted methylation sequencing (Methyl-Seq) in other five pregnancies. Our results showed: 1) a significant hypomethylation in placenta compared to cord blood (including LINE-1, promoters, CpG islands, gene bodies, and tilings); 2) a more pronounced LINE-1 hypomethylation in placenta of small for gestational age neonates; 3) similar methylome profiles among cord blood samples, whereas they were variable in

placenta, suggesting a placental broader plasticity compared to the fetus. Gene-ontology of the 1000 most variable sites between cord blood and placenta showed that promoters and gene bodies that are hypermethylated in placenta are associated with blood specific functions, while those hypomethylated mainly belong to pathways involved in cancer (mainly neuroendocrine). Taken together these evidences support the functional analogies between placenta and cancer.

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P20.042.A Characterization of placental methylation quantitative trait loci

Ariadna Cilleros-Portet[1], *Sergi Marí*[1], *Barbara Paola González-García*[1], *Iraia García-Santisteban*[1], *Mariona Bustamante*[2], *Loreto Santa Marina*[3], *Jose Ramon Bilbao*[1,4], *Nora Fernandez-Jimenez*[1]

[1]*Department of Genetics, Physical Anthropology and Animal Physiology, University of the Basque Country (UPV/EHU) and Biocruces-Bizkaia Health Research Institute, Basque Country, Spain,* [2]*ISGlobal, Barcelona Institute for Global Health, Barcelona, Spain,* [3]*Biodonostia Health Research Institute, San Sebastián, Gipuzkoa, Spain,* [4]*Spanish Biomedical Research Center in Diabetes and Associated Metabolic Disorders (CIBERDEM), Madrid, Spain.*

Introduction: Placenta plays a crucial role in the mother-child interplay during prenatal development, and placental DNA methylation could reflect environmental exposures and affect health and disease in early infancy. However, the contribution of genetics to DNA methylation patterns remains unclear. The aim has been to map placental DNA methylation Quantitative Trait Loci (mQTLs) to ascertain to which extent genetic background shapes the methylation landscape.

Material and Methods: To establish placental mQTLs we combined DNA methylation and genotype data from 373 samples from the INMA cohort. Linear regressions between genotypes and methylation levels were performed using TensorQTL in a ±1 Mb window, adjusted by sex and five principal components. We also annotated the mQTL-participating CpGs and conducted enrichment analyses with the DisGeNET database.

Preliminary results: We obtained 61,105 significant placental mQTLs from which, 39,284 of these were located inside one or more genes. We observed an enrichment of mQTL-participating CpGs in open sea, and CpG island shelf and shore regions, but a significant absence in promoter regions and stable areas of the methylome, such as Partially Methylated Domains (PMDs), and imprinting regions. The mQTL-CpGs presented intermediate methylation (around 60%) compared to the typical trimodal distribution of the placenta (with particular enrichment around 0 and 100% methylation). Finally, the Gene Set Enrichment Analysis showed that the genes located closest to the mQTL-CpGs were enriched in 53 traits, including, colorectal cancer and several drug-dependency and -abuse related traits. Funding: MINECO-PID2019-106382RB-I00 and GV-SAN2018111086 to JRB, ISCIII-PI18/01142 to LSM and GV-SAN2019111085 to NFJ.

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P20.043.B SV detection, SNP phasing and haplotype methylation calling from one nanopore sequencing dataset provides insights to complex genomic disorder

Heather Mary Jeffery, *Philipp Rescheneder, Daniel Turner, Phillip James, Sissel Juul*

Oxford Nanopore Technologies, Oxford, United Kingdom.

Genetic disorders are a major factor influencing human morbidity and can manifest through combinations of genetic, epigenetic and environmental factors. Often, determining the underlying aetiological features of a given disease requires a multi-faceted approach. Genomic disorders which present as both genetically and clinically heterogeneous, like Prader-Willi syndrome, add a further layer of complexity and hinder genetic interrogation at targeted loci.

Here we use Prader-Willi syndrome as an example of how one method i.e. long-read whole genome nanopore sequencing, can provide detailed insights into the underlying genetic and epigenetic causes of a rare inherited genomic disorder.

Prader-Willi syndrome is characterised by the loss of function of usually paternally expressed genes on a ~5Mb region of chromosome 15. We describe how whole genome sequencing, structural variant (SV) detection, SNP phasing and concurrent haplotyped methylation data from one PromethION flow cell sequencing run can be used to characterise the underlying genetic and epigenetic changes of Prader-Willi trios along with their patterns of inheritance.

Within the three trios we investigated, we have examples of i) an SV removing paternally expressed alleles paired with silencing of maternal alleles by methylation, ii) a paternally inherited small SV in the imprinting centre silencing a wide range of the paternally expressed alleles via methylation of promoter regions, and iii) SNP phasing identification of uniparental disomy of maternal alleles leading to complete gene silencing.

Similar combinatorial analysis using nanopore sequencing can also be applied to other complex and/or unresolved genetic disorders to provide cost and time-efficient biological insights.

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P20.044.C Common *RUNX3* missense variant contributes to psoriatic arthritis by modifying differentiation of $CD8^+$ T-cells

Iris Kerker[1], *Sabine Löhr*[1], *Steffen Uebe*[1], *Bernt Popp*[1,2], *Georgia Vasileiou*[1], *John Bowes*[3], *Philipp Kirchner*[1], *Ina Becker*[4], *Emiliano Giardina*[5], *Eleanor Korendowych*[6], *Arif B. Ekici*[1], *Pauline Ho*[3], *Frank Behrens*[7], *Michaela Köhm*[7], *Georg Schett*[8], *Jürgen Rech*[8], *Gunter Assmann*[9], *Ali Nimeh*[10], *Leonid Padyukov*[11], *Gerd-Marie Alenius*[12], *Neil J. McHugh*[13], *Heinrich Sticht*[14], *Benjamin Frey*[4], *Harald Burkhardt*[7], *Anne Barton*[3,15], ***André Reis***[1], *Ulrike Hüffmeier*[1]

[1]*Institute of Human Genetics, University Hospital Erlangen, Erlangen, Germany,* [2]*Institute of Human Genetics, University of Leipzig Hospitals and Clinics, Leipzig, Germany,* [3]*The Centre for Genetics and Genomics, University of Manchester, Manchester, United Kingdom,* [4]*Department of Radiation Oncology, University Hospital Erlangen, Erlangen, Germany,* [5]*University of Rome Tor Vergata, IRCCS Santa Lucia Foundation, Rome, Italy,* [6]*Royal National Hospital for Rheumatic Diseases, NHS Foundation Trust, Bath, United Kingdom,* [7]*Division of Rheumatology and Frauenhofer Institute for Translational Medicine and Pharmacology ITMP, Goethe University, Frankfurt am Main, Germany,* [8]*Department of Internal Medicine 3 - Rheumatology and Immunology, Friedrich-Alexander-Universität Erlangen-Nürnberg and University Hospital Erlangen, Erlangen,*

Germany, [9]Department of Internal Medicine I, José-Carreras Centrum for Immuno- and Gene Therapy, University of Saarland Medical School, Homburg/Saar, Germany, [10]Department of Rheumatology, Fachklinik Bad Bentheim, Bad Bentheim, Germany, [11]Karolinska Institute, Stockholm, Sweden, [12]Department of Public Health and Clinical Medicine/Rheumatology, Umeå University, Umeå, Sweden, [13]Department of Pharmacy and Pharmacology, University of Bath, Bath, United Kingdom, [14]Bioinformatics, Institute of Biochemistry, FAU-Erlangen-Nürnberg, Erlangen, Germany, [15]NIHR Manchester Musculoskeletal Biomedical Research Unit, Central Manchester Foundation Trust and University of Manchester, Manchester Academy of Health Sciences, Manchester, United Kingdom.

Psoriatic Arthritis (PsA) is a chronic T-cell mediated joint disease occurring in up to 30% of patients with psoriasis vulgaris (PsV). Genome-wide association studies identified >60 largely overlapping susceptibility loci for PsA/ PsV with mostly undefined disease-contributing mechanisms. At one of these, the *RUNX3* locus, association has also been described in celiac disease and ankylosing spondylitis. The gene encodes a transcription-factor expressed in T-cells and skin. Insufficient coverage by genotyping-arrays at *RUNX3* prompted a fine-mapping approach using 32 tagging SNPs. Association analysis in 3,049 European PsA patients and 6,178 controls showed significant association to 5 SNPs (6.52E-12≤p≤2.82E-07) within one intragenic LD-block. Genomic annotations for SNPs in high linkage disequilibrium were inconclusive. Haplotype and conditional analyses pointed to disease-contribution by the common variant c.53T>A/p.Ile18Asn. Its proximity to an intron suggested alternative splicing, but transcriptomes in $CD8^+$ T-cells did not confirm this hypothesis. Comparative transcriptome analysis in $CD8^+$ T-cells indicated altered T-cell regulation affecting their differentiation, activation and signaling. In carriers of the risk-allele, the $CD8^+$ T-cells were increased, NK cells decreased; the ratio of $CD4^+/CD8^+$ correlated significantly with the genotype of the disease-contributing variant ($p = 0.035$). Our study indicates that the *RUNX3* risk-allele in PsA affects differentiation of T-cells, a disease-mechanism which might also be relevant for other complex autoimmune diseases.

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P20.045.D Comprehensive profiling of multi-omics features in $CD4^+$ T cells revealed that DNA methylation-mediated regulatory variants contribute to substantial heritability of rheumatoid arthritis

Eunji Ha[1], *So-Young Bang*[2,3], *Jiwoo Lim*[1], *Jun Ho Yun*[4], *Jeong-Min Kim*[4], *Jae-Bum Bae*[4], *Hye-Soon Lee*[2,3], *Bong-Jo Kim*[4], *Kwangwoo Kim*[1], *Sang-Cheol Bae*[2,3]

[1]Kyung Hee University, Seoul, Korea, Republic of, [2]Hanyang University Hospital for Rheumatic Diseases, Seoul, Korea, Republic of, [3]Hanyang University Institute for Rheumatology Research, Seoul, Korea, Republic of, [4]National Institute of Health, Osong Health Technology Administration Complex, Cheongju, Korea, Republic of.

Introduction: Rheumatoid arthritis (RA) is a chronic autoimmune disease, primarily affecting joints. $CD4^+$ T cells have been highlighted as the most relevant cell type to RA pathogenesis by non-coding RA-risk variants on $CD4^+$-specific regulatory elements. This study aimed to examine how RA-specific transcriptomic features in $CD4^+$ T cells are led by RA-risk genetic variants with effects on DNA methylation.

Methods: We generated genomic, transcriptomic, and DNA methylation data of $CD4^+$ T cells from 82 RA patients and 40 controls. RA-specific differentially expressed genes (DEGs), differentially methylated regions (DMRs), and their quantitative trait loci (QTLs) were identified to dissect regulatory sources for DEGs in $CD4^+$ T cells based on individual-level inter-omics correlations. A partitioned heritability enrichment analysis was performed using ancestry-matched RA genetic association results to assess the statistical enrichment of RA heritability in query genomic regions. Result: We identified 2,575 DEGs, re-emphasizing T-cell differentiation and activation pathways. DMRs were preferentially located in T-cell-specific regulatory regions, showing significant correlations with the expression of 548 DEGs. QTLs for expression and methylation were detected in 771 DEGs and 83 DMR-methylation-correlated DEGs, respectively. We observed much larger enrichment of RA heritability in DEG-correlated DMRs with a large number of methylation QTLs than in expression-uncorrelated DMRs or non-DMRs.

Conclusions: Our findings demonstrate that DNA methylation alterations driven by RA-risk variants contribute to expressional changes of RA-specific DEGs in CD4+ T cells. Grants: National Research Foundation of Korea (2017R1E1A1A01076388), Korea NIH (2012-N73006-01;2017-NI73002-02), Korea Disease Control and Prevention Agency (4848-308;4845-301), Hanyang University Institute for Rheumatology Research

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P20.047.B Identification of novel *SCN5A* regulatory regions using a CRISPRi system in hiPSC-derived cardiomyocytes

Adrian Pérez-Agustín[1,2], *Hector Hugo Galvez-Leon*[2], *Sara Pagans*[1,2]

[1]Department of Medical Sciences. Universitat de Girona., Girona, Spain, [2]Biomedical Research Institute of Girona (IDIBGI), Girona, Spain.

Genetic alterations in *SCN5A*, encoding the alpha subunit of the cardiac sodium channel, are associated with cardiac arrhythmias and may lead to sudden cardiac death. Recent findings also suggest that aberrant *SCN5A* gene expression may increase susceptibility to arrhythmogenic diseases, but the regulatory mechanisms of this gene are still not well understood. Here, we perform a CRISPRi-based screening along the topological associated domain (TAD) of *SCN5A* in order to identify novel regions involved in the control of its gene activity and topology. We used a human induced pluripotent stem cell (hiPSC) line in which the expression of a deactivated Cas9 fused to a KRAB repression domain (dCas9-KRAB) is induced upon doxycycline (Dox) treatment. We designed several gRNAs targeting hotspot regions within the *SCN5A* TAD, based on ENCODE and ChIP-seq data from cardiac transcription factors. hiPSCs were differentiated into cardiomyocytes resulting in a homogeneous population of mature beating cells within 30 days. *SCN5A* differential expression analysis of +/-Dox-treated cells using RT-qPCR was performed to identify *SCN5A* regulatory regions. Our results show that targeting *SCN5A* promoter regions leads to 2-fold decreased gene expression and identify novel *SCN5A* enhancer regions within the neighboring *SCN10A* gene. Our data confirm that the CRISPRi screening system is suitable for the precise characterization of *SCN5A* regulatory regions. Further analysis of specific regions with ChIP-seq

techniques and electrophysiological assays may be a launch pad to elucidate novel molecular mechanisms underlying arrhythmogenic diseases. Funding: Agaur-fellowship (AP-A), SAF2015-70823-R (MINECO/FEDER-UE).

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P20.048.C Multi-locus imprinting disturbance in a child with Silver-Russell syndrome and maternal effect gene variants

Sunwoo Lee[1], *Eguzkine Ochoa*[1], *Eamonn R Maher*[1,2]

[1]*University of Cambridge, Cambridge, United Kingdom,* [2]*Cambridge University Hospitals, Cambridge, United Kingdom.*

Congenital imprinting disorders (CID) such as Beckwith-Wiedemann Syndrome (BWS) and Silver-Russell Syndrome (SRS) may be associated with multi-locus imprinting disturbances (MLIDs). Mostly, an underlying cause is not identified, but MLID may be associated with biallelic pathogenic variants in *ZNF57* or in maternal effect genes (MEGs) such as *NLRP2*, *NLRP7*, *NLRP5*, *PADI6* and *KHDC3L*. Here we describe a case of SRS-MLID associated with maternal variants in MEGs.Methylation profiling of 63 genomic regions containing imprinted DMRs was undertaken in a female child diagnosed with SRS and compared to healthy controls. The proband demonstrated significant methylation alterations at 35 DMRs among 63 imprinted DMRs. Further Whole-Exome Sequencing analysis revealed that both proband and the mother harboured a heterozygous PADI6 frameshift insertion located in chr1:g.17401225 [GRCh38/hg38, c.1873dupA, p.G624fs], which has not been previously reported. In addition, two further maternal effect missense variants; PADI6 [chr1:g.17401225, c.1456T>C, p.C486R] and NLRP5 [chr1:g.56027344, c.1111C>T, p.L371F]. Both missense variants were classified as VUSs (PM1, PM2, PP3 and PM1, BP4 respectively). *PADI6* is a component of subcortical maternal complex (SCMC), which has a crucial role in early embryonic development and biallelic *PADI6* mutations were initially described in women with infertility characterised by early embryonic arrest. Our genetic findings in this family could be consistent with the occurrence of MLID in the proband secondary to maternal biallelic *PADI6* mutations (which would imply an increased recurrence risk). However, the interpretation of the pathogenicity of rare missense variants in the absence of previous reports or a functional assay is challenging.

S. Lee: None. **E. Ochoa:** None. **E. Maher:** None.

P20.049.D *SMAD4* gene coding transcripts with alternative 5′ ends as colorectal cancer biomarkers

Tamara Babic[1], ***Sandra Dragicevic***[1], *Marko Miladinov*[2], *Zoran Krivokapic*[2,3,4], *Aleksandra Nikolic*[1]

[1]*Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia,* [2]*Clinic for Digestive Surgery, Clinical Center of Serbia, Belgrade, Serbia,* [3]*Faculty of Medicine, University of Belgrade, Belgrade, Serbia,* [4]*Serbian Academy of Sciences and Arts, Belgrade, Serbia.*

Introduction: Aberrant use of multiple promoters contributes significantly to a global change in transcription, which is recognized as a defining feature of cancer. Changes in alternative promoter activity might lead to alternations in expression pattern of transcripts with alternative 5′ ends, making them potential cancer biomarkers. The aim of this study was to profile the expression of two major transcripts of *SMAD4* gene, a key tumor suppressor for most human tissues, and evaluate their potential as rectal cancer biomarkers.

Materials and methods: Portion of SMAD4-201 and SMAD4-202 among total SMAD4 transcripts was analyzed using quantitative PCR in seven permanent human cell lines and twelve tumor and corresponding healthy tissue samples from patients with rectal cancer.

Results: Cell lines Caco-2, HCT116, DLD-1 and SW480 had a similar portion of SMAD4-201 as non-malignant cell line HCEC-1C (between 35% and 50%), while cell lines SW620 and HT-29 contained very low (less than 10%) and very high (almost 100%) portions of SMAD4-201, respectively. The portion of SMAD4-201 transcript was increased for average 20.6% in malignant in comparison to non-malignant tissue ($p = 0.001$). Transcript SMAD4-201 was undetectable in all analyzed samples.

Conclusions: Alteration in the composition of SMAD4 transcripts can be attributed to change in levels of transcripts other than SMAD4-201 and SMAD4-202. The results obtained for transcript SMAD4-201 in human tumor and non-tumor tissue samples indicate translational potential of this molecule as rectal cancer biomarker. Acknowledgement: This research was supported by the Science Fund of the Republic of Serbia, PROMIS, #6052315, SENSOGENE.

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P20.050.A Tanycytes and Co. A single cell analysis of the brain third ventricle

Maxime Brunner[1], *Federico Santoni*[1], *Fanny Langlet*[2]

[1]*CHUV, Lausanne, Switzerland,* [2]*University of Lausanne, Lausanne, Switzerland.*

Tanycytes are specialized ependymoglial cells lining the wall and the floor of the third ventriclenext to ependymal cells from which they are morphologically distinct. Beside from being a neuralstem cell niche, they are known to be involved in a variety of functions such as metabolismregulators and traffic controllers at the interface between blood and brain. Single-cell RNAsequencing was performed on induced adult mouse tdTomato-positive cells isolated by FACS inthree different metabolic conditions (fed, 12h-fast and 24h-fast mice). Sequencing data were mainlyanalyzed using Seurat where differential gene expression (DGE) analysis was conducted onintegrated data between fed and fasting conditions. Different tools were used for inference of cellpseudotime, RNA velocity and for inference of intercellular network communication.We were able to identify potential sub-populations of ependymal cells as well as known and newspecific markers for tanycyte sub-populations. Pseudotime trajectories showed for the first timeastrogenesis in adult mice from alpha-tanycytes. DGE analysis enlightened the activation of severaltranscriptional pathways of induced cellular stress in fast24 compared to fed condition. Intriguingly,in 12h-fast and 24h-fast, an additional cell cluster expressing genes specific of the suprachiasmaticnucleus (SCN) appeared. Given that no cells from SCN were expected to be targeted by thisexperiment, further investigations are ongoing to clarify this finding.In conclusion, this work shows the power of single cell transcriptomics to analyze the complexity ofheterogeneous neural structures and to understand the interplay among key cell types inenvironment sensing and energy homeostasis.

M. Brunner: None. **F. Santoni:** None. **F. Langlet:** None.

P20.051.B TFAP2B haploinsufficiency as a cause of Chronic Instestinal Pseudo-obstruction

Almira Zada[1], *Bianca M. de Graaf*[1], *Laura Kuil*[1], *Jonathan D. Windster*[1,2], *Marjon A. van Slegtenhorst*[1], *Alice Brooks*[1], *Robert M. W. Hofstra*[1], *Erwin Brosens*[1], *Maria M. Alves*[1]

[1]*Department of Clinical Genetics, Erasmus University Medical Centre, Rotterdam, Netherlands,* [2]*Department of Pediatric Surgery, Erasmus University Medical Centre - Sophia Children's Hospital, Rotterdam, Netherlands.*

Introduction: Chronic Intestinal Pseudo-obstruction (CIPO) is a congenital enteric disorder characterized by severe intestinal dysmotility without mechanical obstruction. Although several genes have been described in the etiology of this disease, the majority of patients have an unknown molecular genetic diagnosis. The Transcription Factor AP-2 Beta (TFAP2B) regulates neural crest cells specification. Pathogenic missense mutations in this gene cause Char syndrome, characterized by characteristic facial features, patent ductus arteriosus and hand abnormalities. However, no gastro-intestinal defects have been reported.

Material and methods: Whole exome sequencing of a CIPO patient revealed a *de novo* heterozygous deletion in *TFAP2B* (*TFAP2B* c.602-5_606delTCTAGTTCCA). To prove pathogenicity of this deletion, an exon trapping-splice site assay was performed. The effect of this deletion on RNA and protein expression levels, was also determined by in vitro assays, and compared to two *TFAP2B* missense variants (c.C706T and c.C898T) found in patients with Char syndrome. Moreover, *tfap2b* crispant and morphant zebrafish were generated, to determine the effect of this gene in vivo.

Results: Our results confirmed that this *TFAP2B* deletion affects RNA splicing, and results in loss of exon 4, leading to the appearance of a premature stop codon. As a consequence, decreased RNA levels and absence of TFAP2B protein were observed. No effect on the expression levels of *TFAP2B* was detected for the two Char missense variants. Loss of *tfap2b* in zebrafish leads to hypoganglionosis and delayed intestinal peristalsis.

Conclusions: *TFAP2B* haploinsufficiency likely underlies CIPO pathogenesis, as this gene seems to be required for intestinal development and function.

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P20.052.C A molecular map of long non-coding RNA expression, isoform switching and alternative splicing in osteoarthritis

Georgia Katsoula[1], *Julia Steinberg*[1,2,3], *Margo Tuerlings*[4], *Rodrigo Coutinho de Almeida*[4], *Lorraine Southam*[1], *Diane Swift*[5], *J Mark Wilkinson*[5], *Eleftheria Zeggini*[1]

[1]*Institute of Translational Genomics, Helmholtz Zentrum München, Neuherberg, Germany,* [2]*Cancer Research Division, Cancer Council NSW, Sydney, Australia,* [3]*School of Public Health, University of Sydney, Sydney, Australia,* [4]*Department of Biomedical Data Sciences, Section Molecular Epidemiology, Leiden University, Leiden, Netherlands,* [5]*Department of Oncology and Metabolism, University of Sheffield, Sheffield, United Kingdom.*

Osteoarthritis is a prevalent joint disease and a major cause of disability worldwide. Despite osteoarthritis having a remarkable burden there is currently no curative therapy. Cartilage degradation is a hallmark of osteoarthritis progression and is characterized by large transcriptomic changes. We aim to decipher the molecular mechanisms of knee osteoarthritis pathology in cartilage tissue. We performed RNA sequencing in paired samples of high and low-grade knee osteoarthritis cartilage derived from 207 osteoarthritis patients. We identified markers of osteoarthtis progression combining gene and transcript-level analysis for the first time. We detected widespread differential expression between low and high-grade osteoarthritis articular cartilage for 9,871 genes, differential transcript expression for 189 genes and differences in transcript usage for 79 genes at 5% FDR in the largest transcriptomic study of osteoarthritis to date. We identified 186 genes showing evidence for alternative splicing and described the individual events for the first time in osteoarthritis. We detected novel transcript-specific markers including *ABI3BP, PTPRE, PRDX1 and GADD45* found in both transcript-level and splicing analyses. Gene-level markers novelly associated with osteoarthritis include *TMEM59L* and *PMCH* as well as lncRNA markers including *TENM3-AS1* and *MYOSLID*. Exploring the enrichment of differentially expressed genes and transcripts we detected terms related to extracellular matrix, metabolism and spliceosome formation as well as new pathways gained from transcript-level analysis including collagen related terms. The impacted pathways serve as potential targets for novel therapeutics.

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P20.053.D Chromatin LINE1 RNAs control the switch from quiescence to activation in human T lymphocytes

Federica Marasca[1], *Shruti Sinha*[1], *Rebecca Vadalà*[1], *Benedetto Polimeni*[1], *Valeria Ranzani*[1], *Elvezia Paraboschi*[2], *Renata Grifantini*[1], *Giulia Soldà*[2], *Stefano Biffo*[1], *Sergio Abrignani*[1], *Beatrice Bodega*[1]

[1]*INGM, Milan, Italy,* [2]*Humanitas University, Milan, Italy.*

T-cell quiescence is actively enforced at transcriptional and translational levels, but its epigenetic maintenance is unknown. We explored the role of LINE1, the largest class of Transposable Elements, in human T-cells. We found that LINE1-RNAs is enfolded in chromatin of naïve $CD4^{+}$T-cells and downregulated through mTORC1 upon activation. Sequencing of chromatin-associated RNA identified hundreds of shorter variants of T-cell activation genes with LINE1 novel exons.LINE1 transcripts in complex with Nucleolin reduce expression of the originating genes hampering H3K36me3 levels. We demonstrated that T-cells depleted of LINE1-RNAs increase their effector responses. LINE1-RNAs reappear in T-cells from mTORC1 hyperactivation genetic disease (Lymphangioleiomyomatosis) patients, treated for life with mTORC1 inhibitors,and in dysfunctional T-cells infiltrating colorectal or lung tumors, where LINE1-RNAs depletion rescue T-cell function.Our study uncovers a novel epigenetic mechanism contributing to enforcement of T-cell quiescence and suggests that LINE1 RNAs abundance is critical for T-cell effector function in physiological and pathological contexts.

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P21 New Treatments for Genetic Disorders

P21.002.C Improving the efficiency correction of mutation c.337delG in the *EGFP* gene during cell cycle synchronization in the G2/M phase

Milyausha Zaynitdinova, *Vasilina Sergeeva, Alexander Lavrov, Svetlana Smirnikhina*

Research Centre for Medical Genetics, Moscow, Russian Federation.

Introduction: Genome editing techniques, in particular, CRISPR-Cas9, could correct a wide range of gene mutations. The classic approach is the use of Cas9 nuclease and single guide RNA, which, when paired, create a DNA double-strand break at desired locus. This break is subsequently repaired by HDR, which is possible only in the G2/M phase of the cell cycle, while the major repairing pathway is NHEJ, which creates additional mutations. One of the approaches to increasing HDR is the use of various agents that synchronize cells in the G2/M phase.

Materials and Methods: ABT-751, nocodazole and vinblastine were chosen as synchronizers. At the first stage, their optimal concentrations for HEK293T cell culture were determined: 560 ng/ml for ABT-751, 50 ng/ml for nocodazole and 10.1 µg/ml for vinblastine. Next, we edited the c.337delG mutation at the exogenous *EGFP* gene in the HEK293T. CRISPR-Cas9 components were delivered as plasmid DNA together with ssODN for DNA repair by lipofection. Editing efficiency was assessed by flow cytometry and TIDER.

Results: According to cytometric analysis, the using of ABT-751, nocodazole, and vinblastine increases the efficiency of correction mutation by 2.5, 1.8 ($p < 0.05$) and 3.9 times ($p < 0.05$), respectively. According to TIDER, the efficiency of correction mutation increases to 17.1% (ABT-751, $p < 0.05$), 3.5% (nocodazole, $p < 0.05$) and 1.5% (vinblastine), compared with the basic level (0.4%).

Conclusions: The efficiency of correcting a single nucleotide deletion in the *EGFP* gene increases by synchronization of cell cycle in the G2/M phase in HEK293T culture, apparently due to the activation of HDR.

M. Zaynitdinova: None. **V. Sergeeva:** None. **A. Lavrov:** None. **S. Smirnikhina:** None.

P21.003.D In vitro evaluation of *DMD* transcripts after Golodirsen treatment of MyoD-converted fibroblasts of patients enrolled in the 4053-101 clinical trial

Rachele Rossi[1,2], *Marc Moore*[1,2], *Silvia Torelli*[1,2], *Pierpaolo Ala*[1,2], *Francesco Catapano*[1,2,3], *Rahul Phadke*[3], *Jennifer Morgan*[1,2], *Jyoti Malhorta*[4], *Francesco Muntoni*[1,2]

[1]*UCL Great Ormond Street Institute of Child Health, London, United Kingdom,* [2]*Great Ormond Street Institute of Child Health Biomedical Research Centre, London, United Kingdom,* [3]*UCL Queen Square Institute of Neurology, London, United Kingdom,* [4]*Sarepta Therapeutics, Inc., Cambridge, MA, USA.*

Duchenne muscular dystrophy is an X-linked, neuromuscular disease caused by dystrophin gene (*DMD*) mutations which result in a substantial reduction or absence of the dystrophin protein. Deletions are the most commonly occurring mutation type, disrupting the transcriptional reading frame, and causing dystrophin loss. Antisense oligonucleotide-induced exon skipping can restore the mRNA reading frame and produce an internally deleted, yet functional dystrophin protein, as Exondys 51™ does in patients with confirmed *DMD* gene mutations amenable to exon 51 skipping.

Golodirsen (formerly SRP-4053) is a phosphorodiamidate morpholino oligomer (PMO) developed by Sarepta Therapeutics, Inc., to target exon 53 of the *DMD* gene. In Study 4053-101, we demonstrated exon skipping and dystrophin restoration in all patients. Some variability of protein restoration was observed in different patients, likely due to the not well-understood mechanism of delivery of PMOs and other factors. Here, we aim to assess the exon 53 PMO-induced skipping in primary cell cultures from these patients.Fibroblasts, from patients enrolled in Study 4053-101, underwent Myo-D induced differentiation and were treated with golodirsen. After screening for exon skipping efficiency in treated patients' cells and in healthy controls, we evaluated the transcript 5'-3' imbalance in treated vs non-treated patient cells by custom FluiDMD cards. To better understand the intracellular RNA dynamics of the deleted and skipped products, we investigated the transcript subcellular localization by BaseScope assay. Our data will be correlated with the previously obtained in-vivo data, to provide a more comprehensive assessment of the response to golodirsen in eligible patients.

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P21.004.A Combination of the histone deacetylase inhibitor valproic acid and stopcodon readthrough therapy produces improved alpha-galactosidase activity in Fabry patient-derived R227X fibroblasts

Halil Dündar, *Gürsel Biberoğlu, Aslı İnci, Ilyas Okur, Leyla Tümer, Fatih Ezgü*

Gazi University Faculty of Medicine, ANKARA, Turkey.

Premature terminations codons (PTCs) in the coding regions of DNA form approximately 30% of gene lesions in human genetic diseases, which result in production of non-functional truncated proteins. Some compounds referrred to as stopcodon readthrough drugs induce ribosomes to readthrough PTCs and restore the function of missing proteins in a variety of genetic diseases by interfering with ribosomal function, which allows translation of some full-length protein. This strategy can be applied to any disease provided that the molecular cause is a primary nonsense mutation. Triamterene was a previously identified stopcodon readthrough drug for the treatment of MPS I-Hurler caused by PTCs. In a previous study we demonstrated the suppression of R227X nonsense mutation by treating the fibroblasts of a male Fabry patient with triamterene. Since PTC bearing transcripts are subject to degradation by nonsense mediated decay, we hypothesize that an increase in gene expression by a histone deacetylase (HDAC) inhibitor in combination with triamterene will lead to a greater increase in enzyme activity than triamtrene alone. For this purpose we treated the fibroblasts with triamterene alone (90 µM) and triamterene in combination with different concentrations of (21, 62, 125, 250, 500, 1000 µg/ml) valproic acid (VPA), which is a HDAC inhibitor. In agreement with our hypothesis, we found a more robust increase in α-galactosidase activity in fibroblasts treated with both drug than in cells treated with triamterene alone where triamterene induced readthrough in combination with 125 and 250 µg/ml VPA yielded 2.5 and 3.1-fold more activity than triamterene did.

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P21.005.B Creation of a joint consortium to treat Kosaki syndrome

Maxime Luu[1], *Alison Foster*[2], *Cristina Aguirre Rodriguez*[3], *Hanna Westergren*[4], *Unai Hernandez Dorronsor*[5], *Itziar Martinez Soroa*[6], *Derek Lim*[2], *Sarah Thompson*[2], *Ann Nordgren*[7], *Sara Bluefeather*[8],

Elise Schaefer[9], Marc Bardou[1], Pierre Vabres[10], Jean-Emmanuel Kurtz[11], Laurence Faivre[12]

[1]Centre d'Investigation Clinique – module plurithématique, CHU Dijon, Dijon, France, [2]West Midlands Regional Genetics Service, Birmingham Women's and Children's NHS Foundation Trust, Birmingham, United Kingdom, [3]Internal Medicine Department, Donostia University Hospital, Donostia-San Sebastián, Spain, [4]Neuropaediatric department, Astrid Lindgren's Children's and Youth's Hospital, Stockholm, Sweden, [5]Pediatrics Department, Donostia University Hospital, Donostia-San Sebastián, Spain, [6]Ophthalmology Department, Donostia University Hospital, Donostia-San Sebastián, Spain, [7]Department of Molecular Medicine, Karolinska University Hospital, Stockholm, Sweden, [8]Peadiatrics, Hervey Bay & Maryborough hospital, Maryborough, Queensland, Australia, [9]Service de génétique médicale, institut médical de génétique d'alsace, Hôpitaux Universitaires de Strasbourg, Strasbourg, France, [10]Centre référence MAGEC, Dijon, France et Inserm UMR1231 GAD, Génétique des Anomalies du Développement, Université Bourgogne Franche-Comté, Dijon, France, [11]Department of medico-surgical oncology and hematology, Strasbourg Cancer Institute ICANS, INSERM U4113, Strasbourg, France, [12]Centre de Référence Anomalies du Développement et Syndromes Malformatifs, FHU TRANSLAD et ERN ITHACA, CHU Dijon, Dijon, France et Inserm UMR1231 GAD, Génétique des Anomalies du Développement, Université Bourgogne Franche-Comté, Dijon, France.

Background: Kosaki overgrowth syndrome (KOGS) is an ultrarare disorder characterized by characteristic facial features, tall stature, skeletal features, hyperelastic thin skin and MRI brain anomalies. Vascular and neurological deterioration may arise. This disorder is due to heterozygous activating variants in *PDGFRB*, also responsible for Penttinen syndrome and infantile myofibromatosis. Imatinib mesylate is a tyrosine kinase inhibitor targeting PDGFR that has been extensively used in chronic myelogenous leukemia as well as in GIST, with a well-known and safe toxicity profile. To date, 5 patients with myofibromatosis or Penttinen syndrome have been treated with Imatinib with encouraging safety and efficacy results.

Material and methods: The creation of a joint and pluridisciplinary consortium for treating KOGS has been proposed to 5 international teams that were put in contact after the article (Foster et al., 2020) was published, either by contact between physicians or through families.

Results: The consortium wishes to use a common protocol and to set up follow-up meetings to optimize the sharing of knowledge around the efficacy and tolerance of Imatinib in KOGS, but also administrative/ethical issues. Taking into account the clinical heterogeneity of the syndrome, some efficacy endpoints should be personalized. To date, the first French patient, aged 55 years, has been treated with progressively increasing dose of Imatinib for 1.5 months and already reports some improvement of his quality of life with no adverse outcome.

Conclusion: The consortium is looking for teams interested in joining this international initiative to improve the collective expertise for the benefit of patients.

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P21.006.C Testing therapeutic strategies in MERRF cell models

***Mariantonietta Capristo[1]**, Valentina Del Dotto[2], Concetta Valentina Tropeano[1], Claudio Fiorini[2], Monica Montopoli[3], Valerio Carelli[1], Alessandra Maresca[1]*

[1]ISNB, Bologna, Italy, [2]University of Bologna, Bologna, Italy, [3]University of Padova, Bologna, Italy.

Introduction: MERRF is a mitochondrial encephalomyopathy caused by mtDNA mutations in the MT-TK gene, always found heteroplasmic with a high threshold for the expression of the pathologic phenotype. These mutations lead to a severe defect in the mitochondrial protein synthesis, impairing mitochondrial complexes activity. We tested some therapeutic approaches in MERRF cell models, to rescue defective mitochondrial function.

Materials and Methods: We used fibroblasts and cybrids carrying different loads of the m.8344A>G mutation to test two different therapeutic approaches: i) the increase of absolute wild type mtDNA molecules, inducing mitochondrial biogenesis by over-expression of PGC1α protein and by NAD^+ donor nicotinic acid treatment; ii) the reduction of mutant mtDNA molecules, stimulating the removal of damaged mitochondria, by chronic rapamycin treatment.

Results: The first approach (i) was effective in slightly increasing mitochondrial protein expression and respiration in the wild type and intermediate-mutation load cybrids and fibroblasts, but were ineffective in high-mutation load cell lines. These results suggest that induction of mitochondrial biogenesis is not sufficient to improve mitochondrial respiration in MERRF cybrids and fibroblasts with high mutation load. The second approach (ii), ineffective in cybrids, induced a slight increase of basal and maximal respiration in fibroblasts with high mutation load, and a significant improvement in fibroblasts with intermediate-mutation load, rescuing completely the bioenergetics defect. Indeed, we observed an upregulation of PGC1α, and consequently increased mitochondrial biogenesis, possibly related to inhibition of mTORC1 and activation of TFEB.

Conclusions: Overall, these results convincingly support the potential efficacy of a rapamycin-based therapy for MERRF.

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P22 Genetic Counselling/Services/Education

P22.001.D Further evidence that the pathogenic variant p.Arg592Trp in the *AARS2* gene is not necessarily lethal

***Tessi Beyltjens**, Katrien Janssens, Geert Mortier*

Center for Medical Genetics, Antwerp University/Antwerp University Hospital, Antwerp, Belgium.

Background: *AARS2* is a nuclear gene encoding the mitochondrial enzyme alanyl-tRNA synthetase. Bi-allelic mutations in *AARS2* are known causes of early-onset cardiomyopathy or late-onset leukodystrophy. Until very recently, the p.Arg592Trp variant, either in the homozygous or compound heterozygous state, has been exclusively and repeatedly described in infants with severe cardiomyopathy or primary pulmonary hypoplasia, both resulting in death before the age of 1 year. Nielsen et al. (2020) however reported one girl who developed dilated cardiomyopathy in her teens.

Case report: We report on a 6-year-old boy who was referred for genetic evaluation because of global developmental delay and a severe autism spectrum disorder. Pregnancy and perinatal course were uneventful. Besides gastro-oesophageal reflux there were no general health problems. Exome sequencing revealed the homozygous presence of the pathogenic variant c.1774C>T (p.Arg592Trp) in *AARS2*. Both healthy parents, who denied consanguinity, were heterozygous carriers. MRI of the brain did not show structural abnormalities or signs of leukodystrophy. An

echocardiogram revealed a borderline thickness of the left ventricular wall (z-score: 2.4). Ophthalmologic examination was completely normal.

Conclusion: We confirm the recent clinical letter by Nielsen et al. reporting that the p.Arg592Trp variant is not always resulting in fatal infantile-onset cardiomyopathy. At the age of 6 years, our proband only presented with neurodevelopmental deficits which are occasionally retrospectively found in patients with late-onset leukodystrophy due to bi-allelic pathogenic variants in *AARS2*. These findings are important for genetic counselling, especially when this variant is found in a prenatal setting or early in postnatal life.

T. Beyltjens: None. **K. Janssens:** None. **G. Mortier:** None.

P22.002.A Hereditary breast and ovarian cancer predisposition management for asymptomatic patient carrier of a *BRCA* mutation

Arianna Bonfanti, *Christophe Cordier*

Synlab, Lausanne, Switzerland.

Introduction: Specific inherited mutations in a *BRCA* gene increase the risk of female breast and ovarian cancers and male breast and prostate cancers. Despite the existence of medical guidelines for the management of an asymptomatic *BRCA* mutation carrier, how to manage cancer risk is frequently unclear and patients experienced a sense of disorientation leading a loss of follow up. The aim of our study is to compare the Hereditary Breast and Ovarian Cancer (HBOC) syndrome management in the world for unaffected *BRCA* carriers.

Methods: An online survey was created and sent to at least 200 healthcare professionals around the world. It contains questions about cancer prevention, control continuum and the implication of Genetic Counsellors in the management of asymptomatic *BRCA* carriers.

Results: 47 health professionals from 13 countries participated to our study. 45 respondents declared that there are guidelines in their own country concerning the management of BRCA carriers. For women are recommended mammograms (100%), MRIs (100%), breast ultrasound (85%), serum CA-125 (61,7%), transvaginal ultrasound (59,6%), chemoprevention (61,7%), bilateral risk-reducing mastectomy (100%) and risk-reducing salpingo-oophorectomy (100%). For men is recommended only prostate surveillance (87,2%). Our study concerns also the frequency, the age of beginning and stopping of surveillance and prevention.

Conclusions: Guidelines for HBOC syndrome management are different in the world. Some countries recommend risk-reducing for cancer prevention and others recommend an early detection. The age of beginning and stopping are also different. It would be necessary that each country has the same guidelines concerning the HBOC syndrome management.

A. Bonfanti: None. **C. Cordier:** None.

P22.003.B Predictive genetic testing - uptake in the Department of Clinical Genetics, Children's Health Ireland at Crumlin and two tertiary cardiac referral centres

Claire Winifred Kirk[1], *Jane Lucy Murphy*[1], *Joseph Galvin*[2], *Catherine McGorrian*[2], *Deirdre Ward*[3], *Terence Prendiville*[4], *Margaret Gallagher*[2], *Helen Connaughton*[3], *Sally Ann Lynch*[4]

[1]*University College Dublin, Dublin, Ireland,* [2]*Mater Misericordiae University Hospital, Dublin, Ireland,* [3]*Tallaght University Hospital, Dublin, Ireland,* [4]*Children's Health Ireland at Crumlin, Dublin, Ireland.*

Introduction: Inherited cardiac conditions (ICC) comprising cardiomyopathies and cardiac ion channelopathies predispose to sudden death. Next generation sequencing has facilitated predictive genetic testing of at-risk relatives. This informs management and is cost-effective as patients who test negative can usually be discharged from cardiac follow-up. We investigated the uptake and demographics of predictive genetic testing in the Department of Clinical Genetics and two tertiary referral centres.

Materials and Methods: Data was collected by interrogation of departmental databases and molecular genetic reports at Children's Health Ireland, Crumlin, the Mater Misericordiae University Hospital and Tallaght University Hospital.

Results: In 207 families where a pathogenic/likely pathogenic variant was detected, 1422 relatives had predictive testing. The mean age at testing was 34 years, median age was 35 years (range 25 days - 90 years). Of the 1067 adults tested, 446 were male (41.8%) and 621 were female (58.2%); of the 355 infants/children tested, 188 were male (53%) and 167 were female (47%). On average, six relatives per family were tested (range 1 - 84); 48% ($n = 682$) of patients tested positive and 52% ($n = 740$) tested negative.

Conclusions: Cascade testing is a prolonged process as evidenced by the fact that one of the cases tested was a 5th degree relative of the proband. However, genetic testing has allowed 740 individuals (and their offspring) to be reassured and discharged from long-term cardiac follow-up. Our data suggests that it can be challenging to encourage male relatives to come forward. (Funded by National Children's Research Centre)

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P22.005.D Incidental findings in cytogenetics - the old new

Valentina Miteva[1,2], *Tsanka Ruseva*[2], *Dinnar Yahya*[1,2], *Marya Levkova*[1,2], *Milena Stoyanova*[1,2], *Mari Hachmeryan*[1,2], *Ilina Micheva*[3], *Lyudmila Angelova*[1]

[1]*Department of Medical Genetics, Medical University - Varna, Bulgaria, Varna, Bulgaria,* [2]*Laboratory of Medical Genetics, University Hospital Saint Marina, Varna, Bulgaria, Varna, Bulgaria,* [3]*Clinic of Hematology, University Hospital Saint Marina, Varna, Bulgaria, Varna, Bulgaria.*

Introduction: Much has been discussed over the "incidental" or "secondary" findings, arising from application of genomic technologies, so it is not a completely new dilemma. Genetic counselling has been dealing with outcomes of *tests* that are not related to the initial indication since conventional karyotyping is used.

Materials and Methods: For a period of 10 years among 1554 bone marrow karyotypes performed on both children and adults with haematological disorders, subsequent follow up was suggested in 9 patients. Cytogenetic analysis of peripheral blood lymphocytes was successfully conducted in 7 of them. Genetic counselling was performed.

Results: Suspected chromosomal aberration based on bone marrow result has been confirmed in 6 patients (0.4%) - two robertsonian translocations (14;21) and (13;14), two monosomies X, one paracentric inversion and a ring 18 chromosome. Three of the findings lead to diagnosis of unsuspected chromosomal disorder and the other three required genetic counselling in first grade relatives at risk with possible further impact on their reproduction.

Conclusion: Incidental findings have always been a feature of medicine. A proper follow up of every abnormal result should be

considered. Depending on the patients' will and the neat collaboration between genetic counsellors and other hospital clinicians a number of families can receive proper genetic care.

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P22.006.A Emerging experiences of working in UK clinical genetics services during the COVID-19 pandemic

***Makaela Jacobs-Pearson**, Aamisha Kyada*

Peninsula Department of Clinical Genetics, Exeter, United Kingdom.

Clinical genetics services offer care to adults, children and families with genetic conditions. The COVID-19 pandemic has caused a number of rapid changes to patient care across the UK. Clinical genetics services have adapted to the ongoing challenges of social distancing and redeployment whilst balancing the safety of colleagues and patients. Many centres have rapidly replaced face to face consultations with video and telephone consultations. We have asked Healthcare Professionals in UK clinical genetics services to provide insight into their experience of working during this unique time. Through assessing this data, we will utilise mixed methods to determine the challenges experienced and how services have adapted and developed resilience to provide the best possible service to patients in the landscape of COVID-19 and beyond.

We invited UK based Clinical Geneticists, Genetic Counsellors, Trainees and Students to participate in this questionnaire.

Preliminary results show excellent engagement with 138 responses to date from a wide number of UK clinical genetics services. The impact of the pandemic on Healthcare Professionals is clear; decreased patient and colleague contact is proving challenging. The adaptations, with increased use of telephone and video consultations, have enabled continued patient care. Additionally, our preliminary data shows substantial impact on job satisfaction. Current data trends have identified what is, and is not, working. We therefore hope this research can be used to help plan future healthcare models to protect staff wellbeing and patient care.

We aim to continue hearing from participants with final results collated and analysed in May 2021.

M. Jacobs-Pearson: None. **A. Kyada:** None.

P22.007.B COVID-19 effect on post-test genetic counselling

***Mari Hachmeriyan**[1,2], Maria Levkova[1,2], Dinnar Yahya[1,2], Milena Stoyanova[1,2], Valentina Miteva[1,2], Lyudmila Angelova[2]*

[1]University Hospital "St. Marina", Varna, Bulgaria, [2]Medical University, Varna, Bulgaria.

Introduction: In an era of pandemics along with the emerging possibilities of telemedicine technologies, traditional model shifts from face-to-face to distant genetic counselling. Post-test counselling is crucial for patients' understanding of genetic testing results. The aim of the study is to evaluate Bulgarian patients' attitude towards post-test counselling choice regarding how they receive their results.

Materials and Methods: We performed a retrospective analysis on 5338 patients' post-testing choices, stratifying them into two groups - 5133 pregnant women undergoing first trimester screening for aneuploidies and 205 patients undergoing conventional karyotyping for reproductive reasons. Ten-month period in 2020y during COVID-19 lockdown was compared with the equal one in 2019y. We applied Chi-squared test.

Results: Results showed that during COVID-19 lockdown in the group of pregnant women, 65.9 % of respondents preferred to obtain their results online, that is significantly higher in comparison with the equivalent period from 2019 ($p < 0.05$). However, difference was not found in the second group. In both periods, the couples with reproductive problems preferred face-to-face disclosure, in contrast with the pregnant women's group with statistical significance. This could be explained by the differences between the tests as well as with the possible higher self-protective behaviour of pregnant women towards infectious diseases. Concerning the outcome of the result- positive or negative, there was no significant preference on the receiving method, regardless of the indication.

Conclusions: Certain patients prefer to receive their results online. This emphasizes on the importance of providing them with post-testing counselling with equivalent quality and value to a face-to-face one.

M. Hachmeriyan: None. **M. Levkova:** None. **D. Yahya:** None. **M. Stoyanova:** None. **V. Miteva:** None. **L. Angelova:** None.

P22.008.C Genetic counseling during COVID19 pandemic. One center experience

***Amir Peleg**, Lena Sagi-Dain*

Lady Davis Carmel Medical Center, Haifa, Israel.

Introduction: Genetics and genetic counseling are an integral component in modern clinical healthcare. The COVID-19 pandemic introduced new challenges in genetic counseling services and promoted healthcare tele-technologies. Advantages of this technologies includes reducing patients travel time to medical centers and waiting time. It promote healthcare in remote areas that lack tertiary health care services. Disadvantages include a decrease in rapport-building, possibilities to interpret non-verbal cues, technical or language difficulties, inabilities to perform physical examination, less access to DNA sampling and privacy concerns.

Materials and Methods: From March 15 through December 31 2020, all scheduled visits were evaluated. Tele-counseling was offered when suitable. All requested documents were e-mailed ahead of the tele-appointment. Tele-consultations were not possible in cases when: a. physical or neurological examinations were necessary. b. abnormal genetic tests results. c. patient request d. technical or language difficulties. Following the tele-consultation, secured e-mails sent to patient and referral doctor presented the consultation results. When required, DNA sampling appointments were scheduled. SMS satisfaction questionnaires sent to patients.

Results: Overall, 2211 consultations were performed. 928(42%) by Tele-counseling. 166(7.5 %) by video and the rest by telephone. Out Of the 990 prenatal consultations, 643(65%) used tele-technologies. 390(32%) of the 1221 postnatal consultations used tele-technologies. In comparison to 35% no-show visits during 2019, no-show visits were reduced to 16% (353). Patient's satisfaction with tele services were high.

Conclusions: This study shows that during the COVID-19 pandemic, tele-health improved Medicare and genetic counseling. Tele-medicine seems to be beneficial in genetic counseling healthcare post the pandemic.

A. Peleg: None. **L. Sagi-Dain:** None.

P22.010.A Parenting a child with Down syndrome: a qualitative study of parental experiences to inform genetic counselling

Ellen H. Steffensen[1,2], *Ida Vogel*[1,2], *Stina Lou*[3,2]

[1]*Dept. of Clinical Genetics, Aarhus University Hospital, Aarhus N, Denmark,* [2]*Center for Prenatal Diagnostics, Aarhus University, Aarhus, Denmark,* [3]*Defactum - Public Health & Health Services Research, Aarhus, Denmark.*

Introduction: Knowledge on the experience of families where a child has Down syndrome (DS) is essential to genetic counselling and family support. However, studies qualitatively assessing the parenting experience in Nordic welfare states are scarce. Here, tax funded healthcare and a comprehensive public social service system likely influences the experience. Therefore, we aimed to explore the experience of parents of children with DS living in Denmark.

Materials and Methods: Semi-structured interviews were carried out with 25 parents of 15 children with a postnatal diagnosis of DS aged 4-12 years. Parents represented diverse sociodemographic backgrounds. Reflexive thematic analysis was applied as analytical approach.

Results: Our findings display parents 'at work' in various aspects of everyday life: as moderators of their child's daily activities; as ambassadors of 'the good life' with DS; and as agents advocating the special needs of their child and family. The latter was expressed, for example, as a concern about declining knowledge on DS among health professionals. Interviews also pointed to the significance of belonging to a community exemplified by the value of guidance in parent groups and by parents' ambitions of their child engaging in a social community, e.g. by going on play dates.

Conclusion: This study describes parents acting in an interplay between family unit and society. Our results are of value to professionals providing counselling on DS and to institutions and policy makers planning support to families. Funding: Aarhus University; Health Research Foundation of Central Denmark Region (A2602); Helsefonden (20-B-0065).

E.H. Steffensen: None. **I. Vogel:** None. **S. Lou:** None.

P22.011.B New Spanish translation of EuroGEMS.org: the ESHG's guide to international educational online resources

Adam P. Tobias[1], *Anna Esteve Garcia*[2], *Irene Esteban*[2], ***Edward S. Tobias***[3]

[1]*University of Edinburgh, Edinburgh, United Kingdom,* [2]*West of Scotland Centre for Genomic Medicine, Queen Elizabeth University Hospital, Glasgow, United Kingdom,* [3]*West of Scotland Centre for Genomic Medicine, Queen Elizabeth University Hospital, University of Glasgow, Glasgow, United Kingdom.*

Introduction: The ESHG's www.EuroGEMS.org guide to international educational online resources has now been visited from 115 countries. The website's design, structure and content have been published in Human Mutation, following peer review (PMID: 32906220). The site's content and links have been kept continuously under review and many links to excellent new resources have been added following suggestions from professionals worldwide. Consequently, the proportion of visits made to the site by returning visitors has grown to 28% of the total. Each EuroGEMS.org page already includes a non-English language resources links section. However, adding non-English language translations of the entire website has been suggested.

Methods: Translation of the website's content into Spanish is consequently now being undertaken, by UK NHS bilingual genetic counselling staff. Subsequent independent verification by bilingual genetics staff elsewhere has been arranged. The largest page has already been translated and the remainder should be completed by May 2021. The new Spanish web-pages will be closely linked to corresponding existing English pages, for search-engine optimisation (SEO) and maximal ease of navigation.

Results and discussion: Spanish is the first language of approximately 480 million people worldwide, predominantly in Mexico (122 million), Colombia (49.4 million), Argentina (44.1 million) and Spain (42.9 million). However, only 4.55% of total visits originate from countries with Spanish as a primary language. Translation may greatly widen the website's readership, especially for individuals with genetic conditions, their relatives and the general public. Future translation into additional languages is also being considered. Comments and suggestions would be welcomed (edward.tobias@glasgow.ac.uk).

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P22.012.C Developing an e-learning tool on medical genetics: APOGeE Project (A Practical Online Genetics e-Education)

Sarra Selatnia[1], *Jonathan Berg*[2], *Jill Clayton-Smith*[3,4], *Maurizio Genuardi*[5], *Martin Krahn*[6,7], *Ute Moog*[8], *Edward Tobias*[9], *Peter Turnpenny*[10], *Johannes Zschocke*[11], *Dorica Dan*[12], *Sofia Douzgou Houge*[3,13], *Laurence Faivre*[14,15], *Raoul Hennekam*[16], *Anne Hugon*[1], *Tjitske Kleefstra*[17], *Andrea Manunta*[18], *Giovanni Mosiello*[19], *Alessandra Renieri*[20,21,22], *Ammi Sundqvist*[23], *Marco Tartaglia*[24], *Zeynep Tümer*[25], *Birute Tumiene*[26], *Dagmar Wieczorek*[27], *Lisenka Vissers*[28,29], *Klea Vyshka*[1,30], *Giuseppe Zampino*[31], *Alain Verloes*[1,32]

[1]*Dept. of Genetics, Assistance Publique-Hôpitaux de Paris - Université de Paris, Paris, France,* [2]*Department of Clinical Genetics, Ninewells Hospital and Medical School, Dundee, United Kingdom,* [3]*Manchester Centre for Genomic Medicine, University of Manchester, Manchester, United Kingdom,* [4]*Manchester Centre for Genomic Medicine, Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom,* [5]*UOC Genetica Medica, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy,* [6]*APHM, Hôpital Timone Enfants, Département de Génétique Médicale, Marseille, France,* [7]*Aix-Marseille Université, Inserm, U1251-MMG, Marseille Medical Genetics, Marseille, France,* [8]*Clinical genetic department, Heidelberg University, Heidelberg, Germany,* [9]*Academic Unit of Medical Genetics and Clinical Pathology, Laboratory Medicine Building, Queen Elizabeth University Hospital, University of Glasgow, Glasgow, United Kingdom,* [10]*Clinical Genetics Department, Royal Devon and Exeter NHS Foundation Trust, Exeter, United Kingdom,* [11]*Institute of Human Genetics, Medical University Innsbruck, Innsbruck, Austria,* [12]*Romanian National Alliance for Rare Diseases RONARD, Zalau, Romania,* [13]*Avdeling for medisinsk genetikk, Haukeland universitetssjukehus, Haukeland, Norway,* [14]*UFR Des Sciences de Santé, INSERM-Université de Bourgogne UMR 1231 GAD « Génétique des Anomalies du Développement », FHU-TRANSLAD, Dijon, France,* [15]*Dept of Genetics and Centres of Reference for Development disorders and intellectual disabilities, FHU TRANSLAD and GIMI Institute, University Hospital Dijon, Dijon, France,* [16]*Department of Pediatrics, Academic Medical Centre, Amsterdam UMC, Amsterdam, Netherlands,* [17]*Department of Human Genetics, Radboud University Medical Center, Nijmegen, Netherlands,* [18]*Department of Urology, University of Rennes, Rennes, France,* [19]*Department of Surgery, Urology and Neuro-Urology, Bambino Gesù Pediatric Hospital, Rome, Italy,* [20]*Med Biotech Hub and Competence Center, Dept of Medical Biotechnologies, University of Siena, Siena, Italy,* [21]*Medical Genetics, University of Siena, Siena, Italy,* [22]*Genetica Medica, Azienda Ospedaliero-Universitaria Senese,*

Siena, Italy, [23]RBU International SBH, Solna, Sweden, [24]Genetics and Rare Diseases Research Division, Ospedale Pediatrico Bambino Gesù, IRCCS, Rome, Italy, [25]Department of Clinical Genetics, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark, [26]Vilnius University Hospital Santaros Klinikos, Santariskiu, Vilnius, Lithuania, [27]Institute of Human Genetics and Anthropology, Heinrich Heine University Düsseldorf, Düsseldorf, Germany, [28]Dept of Human Genetics, Radboudumc, Nijmegen, Netherlands, [29]Donders Institute for Brain, Cognition and Behaviour, Radboudumc, Nijmegen, Netherlands, [30]CERCRID, UMR 5137 "Centre de Recherches Critiques en Droit", Université de Lyon, Lyon, France, [31]Department of Woman and Child Health, Center for Rare Diseases and Birth Defects, Institute of Pediatrics, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy, [32]INSERM UMR 1141 "NeuroDiderot", Hôpital R DEBRE, Paris, France.

To fulfil the objectives of e-Training and e-Learning developments in ERNs, the ERN ITHACA will be launching in 2021 an online and interactive textbook of medical genetics, built with the open-access platform Moodle, will the collaboration of multiple authors of from ITHACA's network and from other ERNs. APOGeE with include sections on biological genetics, formal genetics, medical genetics, both clinically oriented and pathophysiologically oriented approach to genetic diseases, precision medicine, and treatment of genetic diseases. APOGeE will connect with other online knowledge sources. APOGeE is ITHACA's main contribution to the EU strategic objectives of ERN-specific knowledge generation, to contribute to a structured programme of post-graduate education and training in the field of human genetics and Rare Disorders. The aim of this project is to establish a free and open access interactive, asynchronous training source in medical genetics: e-learners will be able to access an interface offering them different blocks of e-learning, self-assessment tools, and monitoring of learning achievement. The original content is coordinated by an international and renowned editorial team. It will be enriched with documents and courses already available in our network. APOGeE targets 1) EU trainees in medical genetics and those in other specialities who are interested in certain chapters of genetics; 2) candidates for the European examination in medical genetics and genomics of the European Union of Medical Specialists (UEMS); 3) MD in training in genetics in less wealthy countries who would have access to a free university teaching tool.

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P22.013.D Reporting uncertain prenatal exome sequencing results: how do medical students handle uncertainty?

Jasmijn E. Klapwijk[1,2], *Marike G. Polak*[2], *Karin E. M. Diderich*[1], *Malgorzata I. Srebniak*[1], *Hennie T. Brüggenwirth*[1], *Stina Lou*[3], *Ida Vogel*[3,4,5], *Vyne van der Schoot*[1], *Iris M. Bakkeren*[1], *Katinka Dijkstra*[2], *Sam Riedijk*[1]

[1]*Department of Clinical Genetics, Erasmus Medical Centre, Rotterdam, Netherlands,* [2]*Department of Psychology, Education & Child Studies, Erasmus University Rotterdam, Rotterdam, Netherlands,* [3]*Center for Fetal Diagnostics, Aarhus University Hospital, Aarhus, Denmark,* [4]*Department of Clinical Medicine, Aarhus University, Aarhus, Denmark,* [5]*Department of Clinical Genetics, Aarhus University Hospital, Aarhus, Denmark.*

Introduction: With the introduction of prenatal Exome Sequencing (ES), uncertainty is often a topic of debate; uncertain results may needlessly burden pregnant couples, whereas withholding results may be needlessly paternalistic. We investigated how medical students handled uncertain prenatal ES results.

Materials and Methods: Fifty-one 5th year medical students participated in a vignette study covering seven uncertain prenatal ES results derived from clinical cases (e.g. incidental and secondary findings). Medical students 1) ranked vignettes on perceived uncertainty, 2) indicated whether they would want to report the results, and 3) indicated how certain they were with their choice to report. Additionally, we investigated students' intolerance of uncertainty, and self-reported anxiety when hypothetical parents indicated to consider termination of pregnancy (TOP) based on the result.

Results: Vignettes that ranked high in uncertainty were reported less often (30/51, 59%) than low uncertainty vignettes (50/51, 98%), $p < 0.001$. Students' self-reported certainty about choices to report was lower for high uncertainty vignettes ($M = 79.99$) than for low uncertainty vignettes ($M = 90.33$), $p < 0.001$. Low or high intolerance of uncertainty was not associated with reporting decisions. Students' anxiety towards TOP considerations of parents was not related to number of reported results, $p = 0.357$ nor to certainty with choices to report, $p = 0.898$.

Conclusions: This study inspected how medical students handled uncertainties from prenatal ES, showing that more uncertain results are less likely to be reported. Additional research is needed to ascertain whether this pattern extends to healthcare professionals in the clinic.

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P22.014.A Identification of familial risk of cardiovascular disease: creating expert-based family criteria for the general population

Tetske Dijkstra[1], *Lieke M. van den Heuvel*[2,3,4], *J. Peter P. van Tintelen*[3], *Christian van der Werf*[4], *Irene M. van Langen*[1], ***Imke Christiaans***[1]

[1]*University Medical Center Groningen, Groningen, Netherlands,* [2]*University Medical Center Groningen;, Groningen;, Netherlands,* [3]*University Medical Center Utrecht, Utrecht, Netherlands,* [4]*Amsterdam University Medical Centers, Amsterdam, Netherlands.*

Introduction: Both inherited and familial cardiovascular diseases can pose a risk of early and preventable cardiovascular events to healthy relatives. Implementing a risk assessment tool to facilitate healthy individuals to evaluate a potential risk of familial cardiovascular disease based on their family history could serve as a solution. However, such family criteria to be used by laymen are non-existent.

Methods: We used a qualitative study design to develop expert-based family criteria to use for risk assessment. In an online focus group with physicians ($n = 7$: cardiologists, clinical geneticists, vascular internist, general practitioners) with expertise in inherited and familial cardiovascular diseases we discussed potential family criteria. These criteria are used as input for a Delphi method to reach consensus among a larger group of expert physicians ($n = 26$).

Results: The focus group resulted in family criteria focussing on cardiovascular events (i.e. sudden death, any cardiovascular disease, implantable cardioverter-defibrillator) at young age in one or more close relatives. Specific cardiovascular diagnoses were considered too difficult for laymen. Additionally, information on referral criteria for familial cardiovascular disease for the general practitioner to act upon a potential risk was recommended.

Conclusions: Preliminary results show that experts prefer to keep the risk assessment tool and thus its criteria as simple as possible to increase usability by laymen whilst remaining critical in advising people to visit their general practitioner because of a potential familial risk. Results of the Delphi method for expert consensus are upcoming. This research is funded by the Dutch Heart Foundation (2019T111).

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P22.015.B An international plan for education, awareness, commemoration and celebration of the July 2022 Bicentennial of Gregor Mendel's Birth in Brno Czechia

Milan Macek Jr.[1], *Maurizio Genuardi*[2], *John J. Mulvihill*[3], *Carla L. Easter*[4], *Daniel Fairbanks*[5], *Susan Lindee*[6], *Jiri Sekerak*[7], *Vojtěch Adam*[8], *Blanka Krizova*[9], *Jakub Carda*[10], *Sarka Pospisilova*[11]

[1]*Department of Biology and Medical Genetics, Charles University - 2nd Faculty of Medicine and University Hospital Motol, Prague, Czech Republic,* [2]*Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy,* [3]*University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA,* [4]*National Human Genome Research Institute, Bethesda, MD, USA,* [5]*Utah Valley University, Orem, UT, USA,* [6]*Department of History and Sociology of Science, University of Pennsylvania, Philadelphia, PA, USA,* [7]*Department of the history of biological sciences, Moravian museum, Brno, Czech Republic,* [8]*Department of chemistry and biochemistry, Mendel University, Brno, Czech Republic,* [9]*Mendel Museum of the Masaryk University, Brno, Czech Republic,* [10]*Augustianian Abbey, Brno, Czech Republic,* [11]*12) Department of Genetics and Genomics, Faculty of Medicine and Center of Molecular Medicine, CEITEC, Masaryk University, Brno, Czech Republic.*

Introduction: Johann Mendel was born July 22, 1822 at Vrazne in Silesia and died as Abbot Gregor Mendel on January 6, 1884 in Brno, Moravia. After eight years of experiments, and another year and a half of compiling and interpreting his results, he founded the science of genetics, giving two lectures at the Natural Science Society in former Brünn (Brno), and publishing two papers on inheritance (1866 and 1870) in the society's journal. But there is much more to his life and work to be seen following his path from a small birth town, to a monastery in Brno, a university in Vienna, political activism in Brno, and, of course, his experimental garden and bee house at the Augustian monastery (mendelmuseum.muni.cz/en).

Methods: To mark the unique occasion, planning has benefited from virtual meetings with an international group representing diverse interests and perspectives, including the ESHG. Mission and Vision: Organize and help implement international programming designed to educate, celebrate, commemorate, and bring awareness to diverse audiences. Increase global awareness among geneticists, other professionals and the public of the current meanings of Mendel's life and work, as a person of science and a cleric, the founder of genetics and contributor to meteorology and agriculture, pedagogy, and his religious community. Goals: Improved science literacy and awareness, education, appreciation of science, celebration, inspiration for trainees, students, and the public, international collaboration, and enjoyment. Brno conference to be held at the Augustinian Abbey: "Bicentennial of the birth of Gregor Johann Mendel", July 20-23, 2022 (www.mendel22.cz)

M. Macek Jr.: None. **M. Genuardi:** None. **J.J. Mulvihill:** None. **C. L. Easter:** None. **D. Fairbanks:** None. **S. Lindee:** None. **J. Sekerak:** None. **V. Adam:** None. **B. Krizova:** None. **J. Carda:** None. **S. Pospisilova:** None.

P22.016.C Well-defined philosophical concepts as a tool for ethical genetic counselling: a complementary course proposal for genetic counsellors' training

Jennifer L. Castañeda

Institute of Mother and Child, Warsaw, Poland.

Genetic counselling is a specific form of medical communication in many aspects. The usual doctor-patient communication in which the doctor informs on diagnosis, therapeutic options, management and prognosis is prone to a rather paternalistic climate and is rarely burdened with ethical dilemmas or value-laden decision-making processes. Genetic counselling, in contrast, involves not only complex information often surpassing the patient's knowledge on health and disease, but also value-laden questions such as parent-offspring disease transmission, pregnancy fate after prenatal diagnosis, genetic testing in late-onset disorders, uncertain prognosis. These aspects undoubtedly require from the counsellor a combination of professional competency with empathic communication skills and the capacity to adequately address ethical issues. The question is whether genetic counselling training meets the need of the latter. An analysis of 50 publications involving ethical aspects of genetic counselling from years 2015 - 2020 was done with the aim of identifying philosophical concepts most frequently mentioned therein. A list of 20 concepts was elucidated including genetic determinism, essentialism, reductionism, as well as philosophical trends: principialism, ethics of care, personalism. With these concepts as a starting point, a description of a possible complementary course on relevant philosophical concepts is proposed with the aim of providing genetic counsellors with adequate philosophical tools for ethical genetic counselling.

J.L. Castañeda: None.

P22.017.D Sudden shift to remote genetic counseling during the COVID-19 pandemic: experiences of genetics professionals

Daniela Turchetti[1], *Linda Battistuzzi*[2], *Benedetta Bertonazzi*[3], ***Lea Godino***[1]

[1]*Division of Medical Genetics; IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy,* [2]*Dipartimento di Informatica, Bioingegneria, Robotica e Ingegneria dei Sistemi, Università degli Studi di Genova, Genova, Italy,* [3]*IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy.*

The COVID-19 pandemic has rendered in-person provision of genetic counseling impossible for prolonged periods in many countries, mandating a sudden shift to remote delivery. We used qualitative thematic analysis to explore Italian genetics professionals' experience with remote counseling. Fourteen group and four individual interviews were conducted after participants had delivered remote sessions. Three themes were identified: 1) technical and logistical issues, 2) communication issues 3) clinical content and outcome of the session. According to participants, not having to travel to the clinic saves consultands' time and expense; however, not sharing a

physical space with consultands and having to rely on technology can negatively impact on effective communication, building trusting relationships and performing accurate psychosocial assessments. Although remote counselling was perceived to favor greater focus and succinct, to-the point communication, participants felt uncomfortable not being able to use visual aids to support the explanation of complex concepts. Demographics and socio-cultural status of consultands emerged as factors influencing the outcome of remote counseling sessions. Anyway, participants felt that gaining more experience with this novel approach would improve their confidence and ability to adapt counseling skills. Based on these findings, we suggest that effective, equitable provision of remote counseling will require an infrastructure able to support videocounseling, sharing of clinical documents and visual aids, and connect with a wide range of devices. Moreover, the structure of sessions should be tailored to the specific requirements of remote counseling and suitable training efforts should be promoted to enhance professionals' communication skills in such setting.

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P22.018.A How do non-geneticist physicians deal with genetic tests? A qualitative analysis

Laurent Pasquier[1,2], *Guy Minguet*[3], *Sylvie Moisdon-Chataigner*[4], *Pascal Jarno*[5], *Philippe Denizeau*[6], *Ginette Volf*[7], *Sylvie Odent*[8], *Grégoire Moutel*[9,2]

[1]*Service de Génétique Clinique, Centre de Référence "Déficiences Intellectuelles de causes rares", Centre Hospitalier Universitaire, Rennes, France,* [2]*INSERM U1086, Anticipe, Normandie Université, Caen, France,* [3]*Institut Mines Télécom Atlantique, Département Sciences Sociales et de Gestion, Nantes, France,* [4]*Laboratoire IODE, Faculté de Droit, Université Rennes 1, Rennes, France,* [5]*Service de Santé Publique, Centre Hospitalier Universitaire, Rennes, France,* [6]*Service de Génétique Clinique, Centre Hospitalier Universitaire, Rennes, France,* [7]*Délégation Régionale Bretagne, Alliance Maladies Rares, Rennes, France,* [8]*Service de Génétique Clinique, CLAD Ouest, Centre Hospitalier Universitaire, Rennes, France,* [9]*Espace régional de Réflexion Ethique, Médecine légale et droit de la santé, Centre Hospitalier Universitaire, Caen, France.*

Genetic testing is accepted to be a common practice in many medical specialties. These genetic tests raise issues such as respect for basic rights, how to handle results and uncertainty and how to balance concerns for medical confidentiality with the rights of third parties. Physicians need help to deal with the rapid development of genomic medicine as most of them have received no specific training on the medical, ethical, and social issues involved. Analyzing how these professionals integrate genetic testing into the patient-provider relationship is essential to paving the way for a better use of genomics by all.

We conducted a qualitative study comprising a series of focus groups with 21 neurologists and endocrinologists about their genetic testing practices in the western part of France. The interviews were transcribed and analyzed for major themes.

We identified an automated care management procedure of genetic testing that affect patient autonomy. The simple fact of having a written consent cannot justify a genetic test given the stakes associated with the results. We also suggest to orient practices toward a systemic approach using a multidisciplinary team or network to provide resources for dealing with uncertainties in interpreting results or situations that require additional technical or clinical skills and, if necessary, to allow for joint consultations with both a geneticist and a non-geneticist medical specialist.

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P22.019.B Integrating genetic professional skills into nursing practice

Efrat Dagan[1], *Sivia Barnoy*[2]

[1]*University of Haifa, Haifa, Israel,* [2]*Tel Aviv University, Tel Aviv, Israel.*

Background and aims: Genetics and genomics are essential aspects of healthcare contributing to precision medical care. In this frame nurses are responsible to undergo continuing education about the potential benefits of genetics and genomics in their routine practice. We aimed at exploring the association of genetic/genomic knowledge, self-epistemic authority (SEA) and perceived importance of genetic/genomic in nursing, and the integration of genetic/genomic skills into nursing practice.

Methods: A cross-sectional study among nurses working in pediatric, obstetric, and internal wards of two medical centers in Israel was conducted between February and October 2018. Participants completed anonymous validated questionnaires. Descriptive statistics and a hierarchical regression model were carried out to determine which variables explained the performance of genetics/genomics practices among participants.

Results: The sample consisted of 423 nurses. The findings demonstrated that although nurses perceived importance of genetics was positive ($M = 2.88$, $SD = 0.68$), and their SEA was average ($M = 2.93$ $SD = 0.75$), low genetic knowledge ($55.05 \pm 14.82\%$) and low integration of genetic skills in nursing practice ($M = 1.90$, $SD = 0.71$) was found. Obstetric nurses had more genetic knowledge, more positive perceptions about genetics, and performed more genetic/genomic skills in their nursing practice.

Conclusions: Although nurses realized the importance of genetics/genomics to their practice, and genetics is part of the Israeli nursing core-curriculum, we found disappointingly low levels of knowledge and performance of genetic skills in nursing practice. The results call for action to establish ongoing genetic education programs for nurses, which would lead to the inclusion of genetics into nursing practice, and prepare nurses to provide personalized medicine.

E. Dagan: None. **S. Barnoy:** None.

P22.020.C Implementing Medical Genetics in Luxembourg: two years after the creation of the National Center of Genetics, overview and perspectives

Arthur Sorlin, *Guillaume Jouret, Christian Müller, Philippe Theis, Karin Dahan, Seval Türkmen, Marizela Kulisic, Nathalie Monhoven, Karin Segers, Arantzazu De Perdigo, Christophe Olinger, Zhi Zhang, Farida Berbache, Daniel Stieber, Dominique Bourgeois, Barbara Klink*

National Center of Genetics, Laboratoire National de Santé, Dudelange, Luxembourg.

Up until recently, the medical discipline of human genetics practically did not exist in Luxembourg and patients were mainly sent abroad. To provide access to the full spectrum of genetic medical care to all individuals living and/or working in Luxembourg, the National Center for Genetics (NCG) at the Laboratoire National de Santé (LNS) was established in April 2018. It is the sole health care provider in the domain of human genetics according to articles 6 and 7 of the Hospital law from 08/03/2018. The NCG also functions as a reference center, with the mission to provide expertise to health

professionals, public authorities, and research partners, develop best practices guidelines and actively participate in national plans. Since its creation, the NCG implemented a multilingual clinical genetic service providing genetic consultations for app. 1,300 patients per year. It provides a broad diagnostic spectrum, from NIPT to hereditary cancer solution, from hemato-oncogenetics to molecular characterization of solid tumors, and coordinates the outsourcing of specific genetic testing to external partner centers when needed. The establishment of a legislative and policy framework as well as the financing model for genetics in Luxembourg are crucial points to address in the future. It will be important to pursue collaborations with international expert centers and networks, in particular the different ERNs. We describe the developments and the implementation of medical genetics in Luxembourg with the challenge to cover all the aspects and diseases in the field of human genetics in a small multilingual country.

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P22.021.D NSGC Prenatal and Cancer MMIC Decision Tools: Patient Reported and Research Outcomes

Barbara B. Biesecker[1], *Susan Christian*[2], *Stephanie Cohen*[3], *Roya Mostafavi*[4], *Jill Slamon*[5], *Karen E. Wain*[6]

[1]*RTI, International, Bethesda, MD, USA,* [2]*Precision Labs, Calgary, AB, Canada,* [3]*Ascension St. Vincent, Indianapolis, IN, USA,* [4]*St. Jude Children's Research Hospital, Memphis, TN, USA,* [5]*Vanderbilt University Hospital, Nashville, TN, USA,* [6]*Geisinger, Lewisberg, PA, USA.*

A key genetic counseling goal is to facilitate informed decision making. The multidimensional model of informed choice (MMIC) was developed as a research outcome to assess decision making in genetic counseling. It is a key outcome of decision interventions aimed at achieving informed choice in personalized medicine. The MMIC is a compound assessment tool with three elements: relevant knowledge; outcome value, and test decision. The relationship among these components differentiates informed choice from less informed choice. Due to the specificity of the MMIC knowledge scale, versions are specific to subspecialties and must represent current information. In addition to a research outcome, the National Society of Genetic Counselors (NSGC) Research, Quality, & Outcomes Committee (RQOC) has prioritized the MMIC as a Patient-Reported Outcome Measure (PROM). As such, it assesses the perceived decision support patients received during genetic counseling. The RQOC developed two knowledge scales for non-invasive prenatal screening and hereditary cancer testing for MMIC decision tools. The objective was to ensure that information key to decision making was current and the literacy level was low for broad accessibility. Plain language was used to develop eight true/false knowledge items deemed critical to understanding. To broaden its accessibility, the scales are being validated in English and Spanish. The NSGC has prioritized the development and promotion of PROMS for assessment of service value and to support reimbursement for clinical services. In countries where reimbursement is less a priority, the MMIC prenatal and cancer scales are well designed for research outcomes of decision support tools.

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P22.022.A Psychological burden of preimplantation genetic testing (PGT) on mothers with multiple monogenic disorders and the role of genetic counselling in Saudi Arabia

Monira AlShehri[1], *Alya Qari*[1,2], *Moeenaldeen AlSayed*[1,2], *Ameera Balobaid*[1,2], *Wafa AlQubbaj*[1,3]

[1]*Alfaisal University, College of Medicine, Riyadh, Saudi Arabia,* [2]*Department of Medical Genetics, King Faisal Specialist Hospital and Research Centre (KFSH&RC), Riyadh, Saudi Arabia,* [3]*Department of Pathology and Laboratory Medicine, King Faisal Specialist Hospital and Research Centre (KFSH&RC), Riyadh, Saudi Arabia.*

Introduction: The interplay between multiple genetic diseases, poor ovarian response, arrested embryonic development, and aneuploidy can have a psychosocial impact. Studies analysing the effect of PGT on mothers with only one genetic disorder revealed that PGT can destabilise individuals.

Materials and Methods: This is a retrospective study of attitudes toward PGT in mothers carrying multiple genetic disorders (n = 31). Phone interviews were conducted with mothers who had undergone PGT during January 2009-March 2020 at King Faisal Specialist Hospital and Research Centre (KFSHRC), Riyadh. Data was analysed using SPSS. This study focused on participants' sociodemographic background, reproductive history, genetic conditions, number of children (healthy/affected), number of IVF cycles, attendance at genetic counselling sessions, donation of hematopoietic stem cells, attitudes towards associated moral issues, embryo cryopreservation and prenatal diagnosis (PND) after PGT.

Results: PGT was provided free of charge to participants; their primary concern was emotional. Multiple genetic disorders increase the risk to embryos. Mothers with mild genetic conditions believed PGT was safer than PND. However, most women who did not meet the acceptance criteria for PGT considered PND. This study revealed that mothers undergoing PGT require further counselling. Genetic counsellors can accurately estimate the risk to embryos. However, the counsellor's role extends beyond merely identifying genetic abnormalities. After failed PGT cycles, sessions must be available for families with multiple genetic diseases to assess future risks.

Conclusion: Patients undergoing IVF/PGT who do not have healthy offspring/alternative reproductive options require customised genetic counselling sessions to clarify their risk of recurrent reproductive problems.

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P22.023.B Kabuki syndrome: case series from one local centre

Akane Akane Kondo Kondo[1], *Nobuhiko Okamoto*[2], *Aki Hayashi*[1], *Mikio Yamasaki*[1], *Mikio Morine*[1], *Kenji Hinokio*[1], *Kazuhisa Maeda*[1]

[1]*Shikoku Medical Center for Children and Adults, Zentsuji, Japan,* [2]*Osaka Women's and Children's Hospital, Osaka, Japan.*

Introduction: Kabuki make-up syndrome (KMS) is a rare condition described by Kuroki and Niikawa in Japanese population in 1981. The aim of this study is looking into the clinical course before the diagnosis to know what made them to visit genetics clinic. Also, after the diagnosis, what sort of social and medical support was provided in countryside of Japan.

Materials and Methods: We looked into the medical record of 5 patients diagnosed as KMS since 2013 till now retrospectively.

Results: Timing of diagnosis was 1y/o, 2y/o, 3y/o, 4y/o, 9y/o respectively. All the cases showed dysmorphic feature, but not suspected as KMS until they visited genetic clinics. All had been followed up as developmental and growth delay including congenital heart defects. As for the support after diagnosis, it has been settled well by medical social worker and local health centre. None of them joined KMS patient support group.

Conclusions: Some older case was diagnosed after grown up. We suspect one factor can be laboratory issues. Most of the congenital genetic conditions had been diagnosed as clinical research at some University or Medical centres but not at diagnostic laboratory in Japan. Ordinary paediatrician was not able to order the genetic test for KMS easily until 2020. Also, there were only few hospitals in Shikoku area to provide genetic service. Even so, they have good social support, however it seems difficult to join KMS patient group due to geographical reason. Remote support group can be the next step for future.

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P22.024.C When Science Communication Meets Medical Education

Kinza Mahmood, *Saeeda Bhatti*

University of Glasgow, Glasgow, United Kingdom.

The Year 2 Student Selected Component (SSC) at the University of Glasgow encourages students to explore medical topics outwith their normal medical school curriculum. Here we feedback on the outcomes of two SSCs with a focus on communicating scientific and medical concepts.

The first SSC was delivered to primary 6 pupils at a school Glasgow in 2020. This SSC was an opportunity for students to learn about the important concepts of haematology and genetics in disease, whilst affording them the chance develop the communication skills required to present this information in different formats. Genetics and Haematology are core elements of the medical school curriculum but also on a basic level link back to the biological systems module of the Scottish School Curriculum of Excellence. Sickle cell anaemia was used to teach children about blood physiology and anaemia and a variety of mediums were used which included interactive activities, videos, and presentations.

The second SSC took a more in-depth view of genetics and focussed on the challenges of communicating genetic information. It was delivered online during lockdown 2021. Here students were introduced to a variety of professionals all communicating genetics. The experts ranged from charities, public engagement professionals and medical professionals from the UK and internationally.

In both SSCs the students learned the importance of thinking creatively and sensitively and to adapt their language and approach depending on the audience. They reported that this taught them to be adaptable in their approach to communicating thereby developing their communication skills.

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P22.025.D MOOC on Bioinformatics in Genomic Medicine (BiG MOOC)

Evan Gouy[1,2], *Kevin Yauy*[3,4], *Anne-Sophie Denommé-Pichon*[5,6], *Emanuelle Génin*[7], *François Lecoquierre*[8], *Alban Lermine*[9], *Robert Olaso*[10], *Jean-François Deleuze*[10], *Sacha Schutz*[11], *Marie de Tayrac*[12], *Aurélien Trimouille*[13], *Guillaume Collet*[14], *Xavier Desplas*[2], *Fabien Hobart*[15], *Amodsen Chotia*[2], *Agata Urbanczyk*[16], *Olivier Palombi*[16], *Pascal Pujol*[17], *David Geneviève*[18], *Laurence Faivre*[19,5], *Damien Sanlaville*[1], *Yannis Duffourd*[5,6], *Andrew Green*[20], *Janna Kenny*[20], *Sally-Ann Lynch*[20], *Sarah Wedderburn*[21], *Helena Carley*[22], *Anne Hugon*[23], *Sarra Selatnia*[23], *Klea Vyshka*[23,24], *ERN-ITHACA Executive Committee, Julien Thevenon*[25], *Alain Verloes*[23,26]

[1]*Service de génétique, Hospices Civils de Lyon, Bron, France,* [2]*Centre de Recherche Interdisciplinaire, Paris, France,* [3]*Institute of Advanced Biosciences, Centre de recherche UGA, Inserm U 1209, CNRS UMR 5309, Grenoble, France,* [4]*SeqOne Genomics, Montpellier, France,* [5]*Unité Fonctionnelle Innovation en Diagnostic génomique des maladies rares, FHU-TRANSLAD, CHU de Dijon, Dijon, France,* [6]*UMR1231 GAD, Inserm – Université de Bourgogne Franche-Comté, Dijon, France,* [7]*UMR 1078, Inserm, Brest, France,* [8]*Laboratoire de génétique, CHU de Rouen, Rouen, France,* [9]*MOABI-APHP, Paris, France,* [10]*CNRGH, CEA, Evry, France,* [11]*Laboratoire de Génétique, CHU de Brest, Brest, France,* [12]*Laboratoire de Bioinformatique et Génétique Computationnelle, Service de Génétique Moléculaire et Génomique, CHU de Rennes, Rennes, France,* [13]*Service de génétique médicale, CHU de Bordeaux, Bordeaux, France,* [14]*Réseau BioInfoDiag INCa, Rennes, France,* [15]*Nipédu, Nipcast, Paris, France,* [16]*Université Numérique de la Santé et du Sport, Grenoble, France,* [17]*Unité d'oncogénétique, Service de Génétique Clinique, CHU de Montpellier, Montpellier, France,* [18]*Service de Génétique Clinique, CHU de Montpellier, Montpellier, France,* [19]*Centre de Référence maladies rares "Anomalies du développement et syndromes malformatifs", centre de génétique, FHU-TRANSLAD, CHU de Dijon, Dijon, France,* [20]*Department of Clinical Genetics, Temple Street Children's University Hospital, Dublin, Ireland,* [21]*NHS Greater Glasgow and Clyde, Glasgow, United Kingdom,* [22]*South West Thames Regional Genetics Service, London, United Kingdom,* [23]*Dept. of Genetics, Assistance Publique-Hôpitaux de Paris - Université de Paris, Paris, France,* [24]*CERCRID, UMR 5137 "Centre de Recherches Critiques en Droit", Université de Lyon, Lyon, France,* [25]*Service de Génétique et Procréation, CHU Grenoble Alpes, Grenoble, France,* [26]*INSERM UMR 1141 "NeuroDiderot", Hôpital R DEBRE, Paris, France.*

The development of technologies like Next Generation Sequencing (NGS) has revolutionized the practices of the Medical Genetics. For medical genetic practitioners, new skills are expected to meet the challenges of tomorrow. The future specialist in medical genetics must understand the different steps that lead from phenotyping to diagnosis, the limits, and pitfalls of NGS. There is a need for training of the genetics residents and practitioners in the concepts of algorithmics, NGS data analysis, statistics, and massive data management. The purpose of this project is to teach the medical genomics concepts essential to the production, analysis, and interpretation of NGS data in the framework of rare disorders and ontogenetic. Launched on French platform FUN MOOC in the first half of 2020, the first session gathered more than 5000 participants of which 400 obtained certificates. 15 speakers participated in the creation of the content in videos and additional resources. 2 webinars and a forum allowed to exchange with learners. With the support of ERN ITHACA and UNESS (Digital University for Health and Sport), the content of the MOOC is currently being revised and translated into English to be launched in mid-2021 in a bilingual French-English version. This new version emphasizes on the manipulation of NGS data for interpretation and use of bioinformatics algorithms. Learners from will be able to learn about this field and share their knowledge for the benefit of patients. This project is co-financed by the Connecting Europe Facility of the European Union, under the Action Number 2018-FR-IA-0184.

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P22.026.A Specialist Medical Genetics Training Requirements Across Europe

Freya Anderson[1], *Ute Moog*[2], *Peter D. Turnpenny*[3], *Feliciano J. Ramos*[4], *Edward S. Tobias*[5], *Laura Pölsler*[6,7], *Yvonne Arens*[8], *Jonathan Berg*[1], *European Certificate in Medical Genetics and Genomics (ECMGG) Exam Committee*

[1]*University of Dundee, Dundee, United Kingdom,* [2]*Heidelberg University, Heidelberg, Germany,* [3]*University of Exeter Medical School, Exeter, United Kingdom,* [4]*University of Zaragoza Medical School, Zaragoza, Spain,* [5]*University of Glasgow, Glasgow, United Kingdom,* [6]*Vrije Universiteit Brussel, Brussels, Belgium,* [7]*Medical University Innsbruck, Innsbruck, Austria,* [8]*Maastricht University Medical Centre, Maastricht, Netherlands.*

Introduction: The rapid evolution of Medical Genetics as a speciality has resulted in significant diversity in training programmes and examination requirements. The Union Européenne de Médecins Spécialistes Section of Medical Genetics (UEMS-SMG) was established in 2013 with the aim of promoting the specialty of Medical Genetics across Europe. In 2017/18 the UEMS-SMG developed the European Certificate in Medical Genetics and Genomics (ECMGG) exam, designed to assess competence as a specialist clinician. Optimal development of this exam requires alignment to the existing training requirements of European countries.

Methods: We approached thirty-three European countries for information about their training requirements, using a questionnaire sent to leading specialists. Data gaps were filled using information from national specialty organisations.

Results: 31/33 countries recognise a specialty called "Clinical", "Medical" or "Human" Genetics. Time spent in specialist training varied, with a minimum time to complete specialist training ranging from 4 to 6 years; with an additional 0 to 4 years of training required after medical registration before entering specialist training. In all countries, training included Clinical and Laboratory practice, although the proportions of these components varied widely. In 4 countries, assessment was by logbook completion. In the remainder, examination requirements varied with a combination of multiple choice written examination ($n = 7$), oral examination ($n = 11$) and clinical examination ($n = 10$). Our study suggests that a collaborative approach to developing a European assessment would help standardise training requirements, assisting mobility of specialists across Europe.

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P22.027.B A national survey of Israeli clinical geneticists and genetic counselors on the transition to remote genetic counseling during the COVID-19 pandemic

Nira Schreyer-Shafir[1], *Amihood Singer*[2], *Rachel Michaelson-Cohen*[3], *Orna Staretz-Chacham*[4], *Ehud Banne*[5]

[1]*Genetic Laboratory, Meuhedet Health Maintenance Organization, Lod, Israel,* [2]*Community Genetics, Public Health Services, Ministry of Health, Jerusalem, Israel,* [3]*Medical Genetics Institute, Shaare Zedek Medical Center, Hebrew University of Jerusalem, Jerusalem, Israel,* [4]*Metabolic Clinic, Soroka Medical Center, Ben Gurion University, Beer Sheva, Israel,* [5]*The Rina Mor Institute of Medical Genetics, Wolfson Medical Center, Holon, Israel.*

The COVID-19 pandemic emerged in November 2019 in China and has spread worldwide since. This pandemic has had substantial influences on all aspects of life. The growing understanding that social distancing is the best way to reduce the chance of COVID-19 contagion, combined with the long-term lockdowns, forced the entire healthcare system to adapt new healthcare methods. Prior to the COVID-19 pandemic, genetic counseling in Israel was almost exclusively an in-person face to face visit. This pandemic led the genetic counseling in Israel to suddenly change format to remote methods (either telephone or video-based counseling).

A 35-question anonymous online survey was sent out using the mailing list of the Israel Society of Clinical Geneticists and the Israel Society of Genetic Counselors. Descriptive statistics and a thematic analysis were used to analyze data. A total of 100 surveys were completed, of which 99 respondents had practiced remote genetic counseling in the COVID-19 pandemic era. The remote counseling raised difficulties which included environmental interferences, problems using the technology modalities, and difficulties involving transferring data. On the other hand, overall, genetic counseling providers giving remote counseling were generally satisfied with this transition. We also found that no-show rates, which have major financial consequences, decreased significantly with the transfer to remote counseling.

These results show that remote genetic counseling, given the appropriate administrative, economic, and legal infrastructure, has benefits even beyond the COVID-19 pandemic. We suggest integrating telemedicine routinely as a possible alternative to in-person genetic counseling.

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P22.029.D Secondary findings of exome sequencing in Russian patients

Olga Mironovich, *Polina Gundorova, Tatyana Cherevatova, Anna Orlova, Viktoria Zabnenkova, Alena Chuhrova, Aleksander Poliakov, Oxana Ryzhkova*

Research Centre for Medical Genetics, Moscow, Russian Federation.

Introduction: In exome and genome sequencing, there is a chance of appearance of secondary findings. These variants are located in genes unlikely related to patients diagnosis. The American College of Medical Genetics and Genomics (ACMG) recommends reporting pathogenic and likely pathogenic variants (PVs) in 69 genes linked with medically actionable diseases. In this study, we estimated the proportion of secondary findings of exome sequencing among Russian patients.

Materials and Methods: in DNA samples of 1285 patients clinical or whole exome sequencing were performed.

Results: Among 1285 Russian patients secondary findings have been identified in 36 cases (3%). In 21 cases (60%) the genes were in ACMG list, and in 15 cases (40%) variants were found in the other genes. Most of the variants were found in genes linked with cancer susceptibility syndromes - 36% (*BRCA1, BRCA2, MSH6, RET, PMS2, RAD50, BRIP1, CHEK2*) and congenital heart diseases - 33% (*DSG2, SCN5A, TNNI3, KCNH2, KCNQ1, TNNC1, TTN*). Variants were also found in *RYR1* (Malignant hyperthermia), *LDL, RPCSK9* (Hypercholesterolemia) and *MEFV* (Mediterranean fever) genes. PVs also were found in genes linked to genetics disease not reported in clinical diagnosis of patients, but having distinct clinical manifestations: EXT2 (Exostoses, multiple), NOTCH3 (Cerebral arteriopathy), SOD1 (Amyotrophic lateral sclerosis), CACNA1A (Episodic ataxia) and FGFR3 (Achondroplasia).

Conclusions: Reporting of secondary findings is important both for determining the tactics of further treatment, and for family consulting. Therefore, it is necessary to discuss the addition into laboratory report of PVs in genes not from the recommended list.

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P22.030.A An innovative e-training tool for the clinical management of NDD, the DefiGame serious game

Anne Hugon[1], *C. Immesoete*[2], *B. Chaumette*[3], *J. Clayton-Smith*[4,5], *C. Cravero*[6], *S. Daugy*[7], *D. Dan*[8], *S. Douzgou Houge*[9,10], *L. Faivre*[11,12], *S. Heide*[13], *RC. Hennekam*[14], *T. Kleefstra*[15], *L. Laporte*[16], *I. Marchetti-Waternaux*[17], *A. Manunta*[18], *G. Mosiello*[19], *S. Peudenier*[20], *V. Des Portes*[21,22], *MP. Reymond*[2], *S. Selatnia*[1], *A. Sundqvist*[23], *M. Tartaglia*[24], *Z. Tümer*[25], *B. Tumiene*[26], *L. Vissers*[27,28], *K. Vyshka*[1,29], *D. Wieczorek*[30], *G. Zampino*[31], *A. Renieri*[32,33,34], *A. Verloes*[1,35]

[1]*Dept. of Genetics, Assistance Publique-Hôpitaux de Paris - Université de Paris, Paris, France,* [2]*DéfiScience, Hospices Civils de Lyon, Lyon, France,* [3]*Reference Centre for Rare Diseases with Psychiatric Manifestation, Psychiatry-Neuroscience University hospital Sainte Anne, Paris, France,* [4]*Manchester Centre for Genomic Medicine, University of Manchester, Manchester, United Kingdom,* [5]*Manchester Centre for Genomic Medicine, Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom,* [6]*Reference Centre for Rare Diseases with Psychiatric Manifestations, APHP.SU - Pitié-Salpêtrière hospital group, Paris, France,* [7]*Association Génération 22, Paris, France,* [8]*Romanian National Alliance for Rare Diseases RONARD, Zalau, Romania,* [9]*Manchester Centre for Genomic Medicine, University of Manchester, Manchester, UK, Manchester, United Kingdom,* [10]*Avdeling for medisinsk genetikk, Haukeland universitetssjukehus, Haukeland, Norway,* [11]*UFR Des Sciences de Santé, INSERM-Université de Bourgogne UMR 1231 GAD « Génétique des Anomalies du Développement », FHU-TRANSLAD, Dijon, France,* [12]*Dept of Genetics and Centres of Reference for Development disorders and intellectual disabilities, FHU TRANSLAD and GIMI Institute, University Hospital Dijon, Dijon, France,* [13]*Reference Centre for Rare Intellectual Disabilities, APHP.SU - Pitié-Salpêtrière hospital group, Paris, France,* [14]*Dept. of Pediatrics, Academic Medical Centre, Amsterdam UMC, Amsterdam, Netherlands,* [15]*Department of Human Genetics, Radboud University Medical Center, Nijmegen, Netherlands,* [16]*French Angelman Syndrome Organisation, Paris, France,* [17]*Valentin Organisation for Carriers of Rare Chromosome Disorders, Paris, France,* [18]*Department of Urology, University of Rennes, Rennes, France,* [19]*Department of Surgery, Urology and Neuro-Urology, Bambino Gesù Pediatric Hospital, Rome, Italy,* [20]*Reference Centre for Rare Diseases, Rare Intellectual Disabilities and Multiple Disabilities, CHRU de Brest, Brest, France,* [21]*DéfiScience National Coordinator, Hospices Civils de Lyon, Lyon, France,* [22]*Neuropediatrics Department, Hospices Civils de Lyon, Lyon, France,* [23]*Swedish national Federation for children and youth with disabilities (Rörelsehindrade Barn och ungdomar), Solna, Sweden,* [24]*Genetics and Rare Diseases Research Division, Ospedale Pediatrico Bambino Gesù, IRCCS, Rome, Italy,* [25]*Department of Clinical Genetics, Copenhagen University Hospital, Rigshospitalet, Denmark,* [26]*Vilnius University Hospital Santaros Klinikos, Santariskiu 2, LT-08661, Vilnius, Lithuania,* [27]*Dept of Human Genetics, Radboudumc, Nijmegen, Netherlands,* [28]*Donders Institute for Brain, Cognition and Behaviour, Radboudumc, Nijmegen, Netherlands,* [29]*CERCRID, UMR 5137 "Centre de Recherches Critiques en Droit", Université de Lyon, Lyon, France,* [30]*Institute of Human Genetics and Anthropology, Heinrich Heine University Düsseldorf, Düsseldorf, Germany,* [31]*Department of Woman and Child Health, Center for Rare Diseases and Birth Defects, Institute of Pediatrics, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy,* [32]*Med Biotech Hub and Competence Center, Dept of Medical Biotechnologies, University of Siena, Siena, Italy,* [33]*Medical Genetics, University of Siena, Siena, Italy,* [34]*Genetica Medica, Azienda Ospedaliero-Universitaria Senese, Siena, Italy,* [35]*INSERM UMR 1141 "NeuroDiderot", Hôpital Robert Debré, Paris, France.*

Défigame is a serious game created by the French Network "DéfiScience" dedicated to rare neurodevelopmental diseases (NDD). Its main objective is to develop screening and aetiological diagnostic practices for NDD. The serious game is designed alongside the help of parents and specialists and it allows medical practitioners to reinforce their knowledge in the diagnostic strategies and care of neurodevelopmental disorders in an interactive way. Défigame is based on computer technologies that combine serious objectives with the playful means inspired by video games. This digital training tool is made available to all general practitioners in order to implement clinical practice recommendations and to coordinate actions of providing adequate healthcare and follow-up of patients. This tool contributes to seeking a diagnosis, to improve the early treatment of NDD and to support the family during a diagnosis of a rare and severe disease. In this game, users take on the role of a general practitioner and monitor the developmental trajectory of four young patients. From the first warning signs, to seeking an aetiological diagnosis, the various scenarios help users gain more in-depth knowledge of the neurological development and understand the role of the general practitioner in coordinating the process. The scenarios are inspired from actual events and have been devised in a manner that is as close as possible to the day-to-day reality of the diagnosis of the care of a patient with NDD. This project is co-financed by the Connecting Europe Facility of the European Union, under the Action Number 2018-FR-IA-0184.

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P22.031.B Tele-consultations at the patient's home in genetics: an expected practice, boosted by the pandemic

Allan Lançon[1], *Amandine Beaudouin*[1], *Estelle Petit*[2], *Geoffrey Bertolone*[1,2], *Amandine Baurand*[1,2], *Salima El Chehadeh*[3], *Claire Kastner*[3], *Laurine Tempé*[3], *Christelle Cabrol*[4], *Chloé Trouvé*[4], *Elise Boucher Brischoux*[4], *Mélanie Berard*[5], *Alexia Burtin*[5], *Bruno Leheup*[5], *Mathilde Renaud*[5], *Axelle Rivière*[5], *Céline Poirier*[6], *Marta Spodenkiewick*[6], *Lola Lissy*[6], *Caroline Sawka*[1,2], *Marion Robert*[1,2], *Christel Thauvin-Robinet*[2], *Elodie Gautier*[2], *Sophie Nambot*[1,2], *Juliette Piard*[4], *Martine Doco-Fenzy*[6], *Elise Schaefer*[3], *Laetitia Lambert*[5], *Laurence Faivre*[1,2]

[1]*Service d'oncogénétique, Centre Georges François Leclerc, Dijon, France,* [2]*Service de Génétique, Centre Hospitalier Universitaire, Dijon, France,* [3]*Service de Génétique Médicale, Hôpitaux Universitaires de Strasbourg, Institut de Génétique Médicale d'Alsace, Strasbourg, France,* [4]*Centre de Génétique Humaine, Centre Hospitalier Universitaire, Besançon, France,* [5]*Service de Génétique Clinique, Centre Hospitalier Universitaire, Nancy, France,* [6]*Service de Génétique, Centre Hospitalier Universitaire, Reims, France.*

Introduction: Genetic consultations are often centralized over a large area, sometimes requiring patients to travel long distances. French legislation requires that results, even negative, be

managed within the framework of a genetic consultation. In certain situations (genetic counselling in particular), a clinical examination is not required. For these situations, teleconsultations at the patient's home seem to be an interesting offer. The COVID-19 pandemic has accelerated the implementation of this type of teleconsultation, and has quickly made it possible to assess patient satisfaction.

Material and method: 2307 patients who benefited from a telemedicine consultation by telephone or videoconference between March and December 2020 from the five genetic consultations of the east of France were asked by e-mail or by post to answer an online satisfaction questionnaire.

Results: 20% responded (80% women, 55% over 40 years old). In 56% of the cases, the consultations were by videoconference, in 56% of the cases for a result report, in 64% of the cases as a replacement for a physical consultation due to the epidemic. The satisfaction rate was 96% (excellent (72%) or good (24%)) with 22% who would have preferred a face-to-face consultation, especially when they live near the hospital. Half of the respondents avoided more than 1.5 hours of transport and 69% avoided taking a day off. Patients were less often accompanied by relatives (43% vs. 61%). There was little change in responses during or outside confinement.

Conclusions: These results encourage the optimization of these practices in the long term.

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P22.032.C Telegenetics: attitudes of genetic counselors and patients in Bulgaria

Mariya Levkova[1,2], *Mari Hachmeriyan*[1,2], *Milena Stoyanova*[1,2], *Valentina Miteva*[1,2], *Lyudmila Angelova*[1]

[1]*Department of Medical Genetics, Medical University Varna, Bulgaria, Varna, Bulgaria,* [2]*University Hospital St Marina, Varna, Bulgaria.*

Telegenetics is a useful tool in an era of globalization and especially in a time of pandemic. The present study aims to explore the attitudes of both Bulgarian genetic counselors and random participants to its usage.We conducted an online survey among 200 randomly selected people, who have not visited a genetic counselor, and 19 genetic counselors from Bulgaria in January 2021. The questionnaire explored the willingness of both groups to participate in distant genetic counseling (DGC), referral reasons and the way they would prefer to conduct DGC. Correlation analysis was applied. Mean age of the participants was 35.4 ± 8.4 years. Most of the people (62%) preferred GC in person. Even 63% of people living within two hours away would choose face-to-face GC, although time saving was the leading reason for DGC choice. There was a negative correlation between advanced age and type of GC ($p = 0.03$) - the elders were more prone to DGC, possibly because of the COVID-19 pandemic. Personal contact was the leading advantage of face-to-face CG, but when it comes to post-testing counseling, 81% people would prefer DGC. Most genetic counselors (68%) would dedicate their time in favor of onsite CG, but 63% already provided DGC and supported its application (79%). The majority of both patients and genetic counselors preferred using a video call for DGC. Although telegenetics has been accepted and applied, especially in a pandemic time, major part of both participants and providers of CG prefer face-to-face communication. DGC is favored for post-testing counseling.

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P22.033.D Telemedicine tools to break down barriers in neuromuscular diseases: Clinical Patient Management System (CPMS) and Telegenetics

Fernanda Fortunato[1], *Marianna Farnè*[1], *Francesca Bianchi*[2], *Marcella Neri*[1], *Gabriele Siciliano*[2], *Valeria Sansone*[3], *Andrea Barp*[3], *Emilio Albamonte*[3], *Gianluca Vita*[4], *Antonio Atalaia*[5], *Teresinha Evangelista*[6], *Francesca Gualandi*[1], *Alessandra Ferlini*[1]

[1]*Unit of Medical Genetics, Department of Medical Sciences, University of Ferrara, Ferrara, Italy,* [2]*Neurology Unit, Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy,* [3]*NEMO Clinical Center in Milan, Milan, Italy,* [4]*NEMO Clinical Center in Messina (NEMO Sud), Messina, Italy,* [5]*Institut de Myologie, Sorbonne Université- Inserm UMRS 974, G.H. Pitié-Salpêtrière, Paris, France,* [6]*Unité de Morphologie Neuromusculaire; Institut de Myologie, Sorbonne Université-AIM, Paris, France.*

Introduction: The development of e-health technologies for teleconsultation and exchange of knowledge is within the mission of European Reference Networks (ERN), including Euro-NMD, the ERN for rare neuromuscular diseases. The Clinical Patient Management System (CPMS) is a web-based platform promoting active collaboration within and across ERNs to discuss patient cases. "Telegenetics" represents an attractive alternative to traditional on-site genetic counseling in light of the non-homogeneous availability of genetic services among countries and the increasing demand of high-level expertise.

Materials and Methods: -To improve the use of CPMS platform among Euro-NMD ERN Italian members, a training course, leaded by two medical doctors, was organized through theoretical and practical virtual lessons, addressing CPMS different applications and functions. Panels were created to share and discuss complex cases.- Telegenetic counselling was offered to adult neuromuscular and cardiac patients in a pilot project, called "TeleNEwCARe". Dedicated questionnaires were conceived in order to assess patient satisfaction.

Results: -13 Italian HCPs joined the CPMS training course and 87 panels of discussion were opened; -A total of 55 patients were enrolled for telegenetic counselling during the first 10 months of the project and questionnaires were collected.

Conclusions: The CPMS project was effective in implementing the platform use, maximizing data sharing among care providers, through virtual panels of discussion. Preliminary data from the TeleNEwCARe project show that remote counselling meets the approval of patients, allowing a confidential relationship with clinicians and an effective sharing of information. *Grant Sarepta Therapeutics (CPMS project). This work is generated within the ERN Euro-NMD*

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P22.034.A Pregnancy termination rate following prenatal diagnosis of chromosomal abnormality

Nina Maric

University Clinical Centre of the Republic of Srpska, Banjaluka, Bosnia and Herzegovina.

Introduction: When a chromosomal abnormality is identified prenatally, parents are faced with the option to terminate the pregnancy. That kind of decision is complex and may depend on several factors, primarily on the risk associated with the chromosomal abnormality and the way it is explained to parents.

Materials and methods: In this study, we looked at parental decisions following prenatal diagnosis of a chromosome abnormality by conventional karyotyping in our department from 2009 to 2014. Genetic counseling was provided for the parents that received abnormal results. The pregnancy termination rate was calculated depending on the type of abnormality and associated risk.

Results: Among 90 pregnancies with chromosome abnormalities, 53 (58,89%) were terminated. Pregnancies with autosomal aneuploidy were terminated in 94,44% of cases. Among pregnancies with sex chromosome abnormalities, 83,33% were terminated, including all of the cases with 47,XXY and half of the cases with 47,XXX karyotype. Pregnancies with marker chromosomes were terminated in 20% of the cases. In cases with unbalanced structural abnormalities, the termination rate was 87,50%. Among pregnancies with a balanced structural abnormality, one with de novo translocation was terminated and all with inherited abnormalities were continued.

Conclusion: The findings indicate that most pregnancies with a severe-risk chromosomal abnormality, as autosomal aneuploidy or unbalanced structural abnormality, were terminated, and those with a low-risk abnormality were continued. However, the termination rate in cases with sex chromosome triploidy is unduly high. Because genetic counseling may greatly influence parental decisions, advocating the training of more genetic counselors is very important.

N. Maric: None.

P22.035.A Transition in rare diseases workshops - different perspective of patients, carers and professionals may add an important value to guidelines of care

Karolina M. Sledzinska[1], *Jolanta M. Wierzba*[1], *Tomasz Grybek*[2], *Katarzyna Milska*[1], *Kacper Kowalski*[3], *Joanna Kulisiak-Kazimierczak*[4], *Katarzyna Świeczkowska*[3]

[1]*GUMED, Gdansk, Poland,* [2]*FBB, Gdansk, Poland,* [3]*PSONI, Gdansk, Poland,* [4]*DSW, Wroclaw, Poland.*

Introduction: Rare diseases (RD) with its genetic background may affect up to 6-8% of the Europeans. As the life span of RD patients has recently increased, an issue of care during transition from pediatric to adult care has arisen. To better understand aspects of transition (so far there are no international guidelines available), Rare Disease Centre in Gdansk, Poland, organized workshops for patients and carers (P&C) and medical professionals (MP).

Materials and methods: Through the application process we chose 6 groups (6 people each): 3 MPs and 3 P&C. We asked every group 3 questions regarding challenges of transition that people with disabilities/the families of people with disabilities / specialists must face in adolescence. Then, we mixed groups (MP+P&C; 6 people each) to choose 3 most valuable MP's and P&C's answers separately. At the end, we asked participants to find solutions: imaginary ideal ones and real, possible to implement here and now.

Results: Interestingly, the answers of MPs and P&Cs were different. MPs focused on problems with education, difficulties in finding adequate medical care, random selections of specialist with not enough experience and knowledge needed, issues of family support, legal aspects of care. On the other hand, P&Cs addressed problems of infantilism, not adequate attitude towards adolescents, no independence, sexuality as taboo, lack of self-deciding, including range of medical care needed.

Conclusions: It is important to create guidelines of transition care together with P&Cs, as their expectations towards transition may significantly differ from MPs' view.

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P22.036.C Evaluation of nursing and midwifery capacity to deliver genomic healthcare in Wales United Kingdom

Joanne Elizabeth Swidenbank[1], *Emma Tonkin*[1], *Maggie Kirk*[1], *Siva Ganesh*[1], *Deborah Lancastle*[1], *Mark Davies*[1], *Alexandra Murray*[2], *Michaela John*[2], *Rebecca Hopes*[2]

[1]*University of South Wales, Treforest, United Kingdom,* [2]*Genomics Partnership Wales, Cardiff, United Kingdom.*

Introduction: Nurses and midwives are the largest professional group within the NHS, numbering 32,927 and making up 42% of the NHS health workforce in Wales in 2018. They will play a significant role in delivering genomics-based care to patients and their families. To benchmark the current capacity of these professionals to deliver genomic healthcare, an online survey (available in English and Welsh) was developed. Ethical review and approval University of South Wales [19ET1101LR]. The survey assessed four broad areas: awareness of national and UK-wide genomics initiatives and attitudes to genomics; current professional practice and genomics competency; genomics and the workplace (including support from colleagues and work environment); and influencing factors. Demographic data including clinical role and (regional) health board was also collected along with free text responses.

Materials and Method: Data collection occurred in two rounds between March 2020 and January 31st, 2021. Links to the survey were disseminated though each health board and trust in Wales and via social media.

Results: A total of 262 responses were received from nurses (n =226, 86.1%) and midwives (n = 36, 13.7%) from six out of seven health boards across Wales. Results are currently being analysed. Key findings and relationships within the data will be presented.

Conclusions: These data will be used by Genomics Partnership Wales to inform strategic workforce development and planning of education and training for the Welsh Nurses and Midwives. Funding: KESS MAXI 21434

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P23 Ethical, Legal and Psychosocial Aspects in Genetics

P23.002.B Relatives and providers perspectives on a decision support intervention for cascade screening for beta-thalassaemia major in Pakistan

Hussain Jafri[1], *Mushtaq Ahmed*[2], *Yasmin Ehsan*[1], *Shabnam Bashir*[3], *Yasmin Rashid*[4], *Shenaz Ahmed*[5]

[1]Thalassemia Society of Pakistan, Lahore, Pakistan, [2]Yorkshire Regional Genetics Service, Leeds NHS Teaching Hospitals Trust, UK, Leeds, United Kingdom, [3]Punjab Thalassaemia Prevention Project, Lahore, Pakistan, [4]Health Department Punjab, Lahore, Pakistan, [5]Leeds Institute of Health Sciences, Leeds, United Kingdom.

Introduction: To develop and assess the acceptability of a 'decision support intervention for relatives' (DeSIRe) of children with beta-thalassemia major to facilitate informed decision-making about cascade screening in Pakistan.

Materials and Methods: The DeSIRe was developed by a multidisciplinary team using the International Patient Decision Aids Standards quality criteria and the Ottawa Decision Support Framework. Twelve focus groups were conducted in six cities in the Punjab province of Pakistan: six with relatives of children with βeta thalassaemia major (β-TM) and six with HCPs affiliated with the government funded 'Punjab Thalassaemia Prevention Project' (PTPP).

Results: 117 participants (60 HCPs and 57 relatives) generally valued the DeSIRe for improving understanding of β-TM, thalassaemia carriers and genetic inheritance. It was considered accessible for people with varying levels of education, although some had difficulties understanding the key concept used in genetic counselling, such as, 'genes' and 'inheritance'. Participants also agreed the DeSIRe was pro-choice and suggested using more directive language.

Conclusion: Cultural preferences for using directive language to support decision-making raise ethical challenges for developing interventions using Western theories, frameworks and guidelines that emphasise the importance of non-directiveness. Nevertheless, use of the DeSIRe by HCPs in the PTPP to support decision-making about cascade screening is feasible and acceptable. The findings will inform refinement of the DeSIRe and plans for implementation in the clinical setting.FUNDER: Medical Research Council, Grant Ref: MR/T003782/1

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P23.003.C Development of biobanking in Russia: legal aspect

Iurii S. Povarov, *Andrey A. Inyushkin, Elena S. Kryukova, Valentina D. Ruzanova*

Samara National Research University, SAMARA, Russian Federation.

Introduction: The development of biobanking makes it possible to conduct large-scale population studies, search for biomarkers, and develop new drugs.

Materials and Methods: The Russian legislation accompanying the functioning of biobanks was systematically analyzed.

Results: The legal regime of the biobank as a complex object consists of the regimes of biomaterials and information collected on their basis specified in the law. The main problem in Russia is the disunity of biobanks, which increases the risks, prevents the exchange and rapid use of information for scientific research. The formation of a system of interaction between organizations engaged in biobanking can be carried out by improving the legal regime of biobanks, including in terms of the exchange of biomaterials and relevant information, the formation of registers of genetic data, and strengthening state control over their activities.

Conclusions: It is necessary to formulate general provisions on the status of subjects with biobanks and the regime of bioobanks and the information obtained on their basis on the basis of a single conceptual framework. It is also necessary to single out the procedure of cross-border exchange for the full functioning of biobanks, ensuring autonomous, unified, from the point of view of international rules, legal regulation.The study was carried out with the financial support of the Russian Federal Property Fund in the framework of the scientific project no. 18-29-14073.

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P23.004.D Rapid Genomic Testing in critically ill children: Managing risk and uncertainty during the testing cycle

Isabelle M. Delon[1], *Angus J. Clarke*[2], *F Lucy Raymond*[3]

[1]East Genomic Laboratory Hub, Cambridge University Hospitals, Cambridge, United Kingdom, [2]School of Medicine, Cardiff University, Cardiff, United Kingdom, [3]Department of Medical Genetics, University of Cambridge, Cambridge, United Kingdom.

Over the last five years, rapid genomic testing for critically ill children has been implemented around the world and delivered many successes of life-saving diagnosis demonstrating the clinical utility of the approach. The lack of phenotypic specificity in the neonatal period combined with ever-growing knowledge of gene-disease associations but incomplete understanding of the natural history of disorders in the neonatal period has oriented the diagnostic search towards the agnostic analysis of whole genome sequencing data to increase the chance of a diagnosis. The resulting testing approach is hypothesis-generating rather than hypothesis-testing and we propose represents the epistemology described in Biesecker, 2013 (Genomes Research 23:1051-1053) and a change of paradigm in genomic testing. This new testing model in turn presents many new risks and uncertainties for the research, scientific and clinical teams, through pre- and post-test counselling, testing method, result interpretation, use of the results in clinical management and health systems functions and policies. We explore and deconstruct the risks and uncertainties debated in the literature through the lens of the social construction of knowledge, of frameworks of knowledge and of probability of outcome, and concepts of genomic uncertainty. We discuss the challenges ahead of the community to harness the opportunities offered by novel genomic testing methods in critically ill children.

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P23.005.A Factors that influence data sharing through data sharing platforms: a qualitative study on the views and experiences of cohort holders and platform developers

Thijs Devriendt[1], *Pascal Borry*[1], *Mahsa Shabani*[2]

[1]KU Leuven, Leuven, Belgium, [2]UGent, Gent, Belgium.

Introduction: Data infrastructures are being developed to enhance and facilitate the sharing of cohort data internationally. However, empirical studies have shown that many barriers can impede broad sharing data. Our aim is to describe the barriers and concerns that cohort holders might have over the sharing of cohort data in greater contextual depth, and the implications for data sharing platforms.

Materials and Methods: Seventeen participants involved in developing data sharing platforms or tied to cohorts that are to be submitted to platforms were recruited for semi-structured interviews to share views and experiences regarding data sharing.

Results: Credit and recognition, the potential misuse of data, loss of control, lack of resources, socio-cultural factors and ethical and legal barriers were identified as elements that influence decisions on data sharing. Based on argumentation underlying

restrictions, core values were classified as equality, reciprocity, trust, transparency, gratification and beneficence.

Conclusions: Data generators might use data sharing platforms primarily for collaborative modes of working and network building, such as to find similar cohorts. Data generators might be unwilling to contribute and share for non-collaborative work, or if no financial resources are provided for sharing data. This publication is part of a project that has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 825903. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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P23.006.B Your DNA, Your Say: the views of Italian lay public on sharingDNA and medical information

Virginia Romano[1,2]

[1]*Uppsala University, Uppsala, Sweden,* [2]*Lund University, Lund, Sweden.*

The "Your DNA, Your Say" project is a global online survey gathering lay public attitudes toward access and sharing of DNA and other medical information (Middleton et al., 2018). The survey has been translated into 15 languages and conducted in 22 countries. We report results obtained in Italy regarding trust in a set of selected social entities and willingness to share medical and DNA information (WTS) to these actors.We obtained responses from 1229 persons and performed multivariate correlation to analyse, among others i) trust as distributed per age ii) geographical distribution of WTS and differences amongst the different potential recipients of sharing iii) trust and WTS as related to religiosity. The most trusted social actor and potential data recipient was the category of "My Medical Doctor" which scored an average of 75% across all age ranges. Above and beyond a general positive attitude towards sharing, respondents were clear when expressing their preferences between potential sharing recipients, "My Medical Doctor" (60%) as opposed to "For Profit Researcher" (38%). Finally, the relationship between WTS and religiosity is complex; while religious people seem less inclined (58%) than non-religious (62%) towards sharing with "My Medical Doctor" this trend is overturned when considering sharing with "For Profit Researcher" (religious 41% vs non religious 32%). These survey results suggest that efforts to collect and share genomic data should consider and adapt to the social distribution of trust and willingness to share in the population, and that further research should explore the specific social and cultural contexts of genomic data.

V. Romano: None.

P23.008.D A couple based approach to expanded carrier screening in a fertility setting

Juliette Schuurmans[1,2], *Mirjam Plantinga*[1], *Angela Fenwick*[2], *Irene van Langen*[1], *Anneke Lucassen*[2]

[1]*University Medical Center Groningen, Groningen, Netherlands,* [2]*Clinical Ethics and Law, Faculty of Medicine, University of Southampton, Southampton, United Kingdom.*

Introduction: Approximately 1 in every 100 couples are at risk of a child with an autosomal recessive condition. Often this carrier couple status will not be known until the birth of an affected child, yet new technologies facilitating the rapid simultaneous testing of many different carrier states- expanded carrier screening (ECS)- now mean those couples at risk can be identified before they conceive. We previously described how GPs in the Netherlands met criteria for responsible implementation when offering this test to their patients. Instead of reporting individual carrier states, results were provided as a couple-result. Here, we describe the offer of ECS couple-testing in a fertility setting, where those seeking fertility treatment may not both be providing the gametes to be used in conception.

Methods: We explored healthcare professionals' (HCPs) (6 focus groups) and couples' views (14 interviews) regarding the ethical issues and implications of couple-based ECS in a UK-based fertility setting. Data were analysed thematically. Findings: Couples and HCPs were supportive of the concept of couple-results. However, many participants found this a difficult concept to grasp and often reverted to discussing individual results. HCPs recognised the limited utility of disclosing individual carrier results, but thought that their responsibilities to report these might be different in this setting, where the social and 'genetic' couple are sometimes different.

Conclusion: Although the general population and fertility arms of the study are not directly comparable (and took part in different countries), both shed important light on perceptions around individual and couple test results.

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P23.009.A Why do Belgian reproductive-aged women choose to accept or decline expanded carrier screening?

Eva Van Steijvoort[1], *Remke Demuynck*[1], *Hilde Peeters*[2], *Hilde Vandecruys*[3], *Jasper Verguts*[3], *Karen Peeraer*[4], *Gert Matthijs*[5], *Pascal Borry*[1]

[1]*Department of Public Health and Primary Care, Centre for Biomedical Ethics and Law, KU Leuven, Leuven, Belgium,* [2]*Department of Human Genetics, KU Leuven, Leuven, Belgium,* [3]*Jessa Ziekenhuis Hasselt, Hasselt, Belgium,* [4]*Department of Development and Regeneration, Woman and Child, KU Leuven, Leuven, Belgium,* [5]*Department of Human Genetics, Laboratory for Molecular Diagnosis, KU Leuven, Leuven, Belgium.*

Introduction: Expanded carrier screening (ECS) allows to identify future parents at risk of conceiving a child affected with an autosomal or X-linked recessive monogenic condition. A Belgian carrier screening offer for reproductive partners became available in October 2019.

Materials and Methods: Non-pregnant women visiting their gynaecologist were invited to complete a questionnaire assessing socio-demographic characteristics, the perceived susceptibility of being a carrier/conceiving a child with a hereditary condition, the acceptability of offering ECS, attitudes towards ECS, the intention to participate in ECS and reasons to accept or decline ECS.

Results: Most women (n = 127) were between 25-34 years old (60%), in a relationship (91%) and wanted to have children in the future (65%). Being able to share genetic information with children or relatives (82%), to prevent the birth of a child affected with a hereditary condition (81%) and to know the risk of conceiving a child with a hereditary condition (80%) were the main reasons to accept ECS that were selected by the majority of women. The most common reasons for declining ECS were the possible concerns that could arise when receiving test results (21%), having no family history of hereditary disorders (15%) and not wanting to take action based on test-results (before or during pregnancy) (10%).

Conclusions: Our findings show that potential users of ECS in Belgium show positive attitudes towards ECS and would consider

participating in ECS in the future. The results of this study can be used to develop pre-test counseling services. Grant references: Research Fund Flanders (FWO).

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P23.011.C What specialists talk about medical genetics in Russia? (based on a results of expert interviews)

Alexander Yu. Dolgov[1,2]

[1]*Institute of Scientific Information for Social Sciences of the Russian Academy of Sciences (INION), Moscow, Russian Federation,* [2]*HSE University, Moscow, Russian Federation.*

Introduction: The aim of the study is to find out how experts look at the growth of popularity of genetics, how they describe the expectations and requests from the government and society, what problems, in their opinion, they face in the development of scientific knowledge in Russia.

Methods: Expert interviews (N = 13) were conducted with Russian specialists in the field of human genetics, medical genetics, and genomic medicine.

Results: The following main topics were highlighted: uncertainty; government support and regulation; the professional community problems; ethical limitations and responsibility to patients; expectations, fears and prejudices of people. The problem of uncertainty is one of the key issues for the current stage of knowledge about human genome. Government's interest in the results of genetic research plays a controversial role. On the one hand, government support measures are being improved, and on the other hand, excessive regulation of scientific activities appears, while many issues remain unresolved in terms of their legal regulation. There is also a problem in the academic community at the level of interaction with doctors who are not work with genetic data. Experts call ethical limitations and responsibility to patients the main principles of their work. In the experts' statements, professional ethics is a working self-regulatory mechanism. Finally, experts noted that people are not ready to introduce genetic technologies into their everyday lives. The study was supported by the Russian Science Foundation (project № 19-18-00422).

A.Y. Dolgov: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Russian Science Foundation.

P23.012.D Legal issues relating to genome editing in human embryos: lessons from the French Bioethics Law

Anastasia Constantin[1,2], *Bettina Couderc*[1,2,3], *Emmanuelle Rial-Sebbag*[1,2]

[1]*CERPOP, UMR 1295, Inserm, Université de Toulouse - Université Toulouse III Paul Sabatier, Toulouse, France,* [2]*Plateforme "Ethique et Biosciences" (Genotoul Societal), GIS Genotoul, Toulouse, France,* [3]*Institut Claudius Regaud - IUCT-Oncopole, Toulouse, France.*

France has its own vision of bioethics and thus has adopted specific laws on bioethics covering advances in biology and medicine. The laws are periodically revised regarding scientific advances and societal demands. The first laws were adopted in 1994, revised in 2004 and 2011. They are currently under revision (to be adopted by the end of first semester 2021). Several fields are covered: human research, donation and use of elements and products of the human body, medically assisted procreation, genetics and genome manipulation, use of personal health data for research purposes and neurosciences. The recent discovery of the new genome editing biotechnology called CRISPR-Cas9 has raised huge debates in this revision process. Developed by E. Charpentier and J. Doudna, CRISPR-Cas9 enables targeting and cutting specific DNA segments in the genome to remove or add few nucleotides or substitute another DNA sequence. Compared to previous technologies, it is more reliable, easier and cheaper to use; even if serious technical problems still exist. The ethical/legal discussions that have occurred, in particular regarding genome editing in human embryos, tackled questions on its use in research and in clinical applications. The French bioethics law still firmly forbids the clinical application, however the use of genome editing in human embryos for research purposes is debatable in the French Parliament. The French bioethics law, its current revision and the related legal issues with genome editing in human embryos will be presented. *Supported by ANR as part of the ComingGen project n°ANR-18-CE38-0007*

A. Constantin: None. **B. Couderc:** None. **E. Rial-Sebbag:** None.

P23.013.A The efficacy of genetic counselling for familial colorectal cancer. Findings from a randomised controlled trial

Andrada Ciuca[1], *Adriana Baban*[1], *Tara Clancy*[2,3], *Ramona Moldovan*[1,2,3]

[1]*Department of Psychology, Cluj-Napoca, Romania,* [2]*Manchester Centre for Genomic Medicine, Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom,* [3]*Division of Evolution and Genomic Sciences, School of Biological Science, University of Manchester, Manchester, United Kingdom.*

Background: Genetic counselling (GC) for familial colorectal cancer (fCRC) has been shown to improve outcomes such as emotional distress and screening adherence. This is the first randomised clinical trial to evaluate the efficacy of GC for fCRC.

Method: We included individuals affected or at risk for fCRC (Lynch syndrome, APC-associated polyposis, MUTYH-associated polyposis or clinically defined fCRC). Participants were randomised to (1) genetic counselling and standard care or (2) standard care alone (control). Measures include empowerment (Genetic Counselling Outcome Scale, GCOS), knowledge, risk perception, emotional distress, screening/surveillance behaviours, perceived social support, decisional conflict and quality of life.

Results: We currently recruited 56 participants. The average age of participants is 46 years old, with 50% females. Our data indicate a significant effect in terms of empowerment ($p = 0.0002$), depression ($p = 0.03$), anxiety ($p = 0.03$) and knowledge ($p = 0.01$), when comparing the genetic counselling group with the control group, at post-intervention. Anxiety and depression at baseline had a moderating effect on the change in empowerment. Participants scoring lower on anxiety ($p = 0.009$) and depression ($p = 0.011$) at baseline had a greater increase in empowerment scores after genetic counselling.

Conclusions: Our data shows significant improvements for both the primary endpoint (empowerment) and secondary endpoints (knowledge, depression, anxiety). We hypothesize that these trends will be maintained and our findings will be further supported by the sample ($n = 68$) we intend to recruit to ensure power for statistical and clinical significance.

A. Ciuca: None. **A. Baban:** None. **T. Clancy:** None. **R. Moldovan:** None.

P23.014.B Genetic counsellors and legal recognition: a made-for-Canada approach

*Dimitri Patrinos[1], **Deborah M. Lambert**[2], Bartha Maria Knoppers[1], GenCOUNSEL Study, Ma'n Zawati[1]*

[1]Centre of Genomics and Policy, McGill University, Montreal, QC, Canada, [2]National Rare Diseases Office, Dublin, Ireland.

Genetic counselling is a fast-growing profession in Canada, but despite this growth, is only recognized legally in 1 of 13 Canadian provinces and territories. Legal recognition ensures safety in the provision of healthcare services by regulating professions that are considered to pose a risk of harm to the public, if not properly regulated. It also offers title protection to legally recognized professionals.

We surveyed a newly-formed special interest group for provincial genetic counselling regulation and estimate that there are 484 individuals in Canada to be regulated as genetic counsellors (n = 484), with 89% found in only 4 of 13 jurisdictions. Compared to other regulated professions, the route towards legal recognition for genetic counsellors may be challenging due to its small number of practitioners. Under Canadian law, there are three models of legal recognition: 1) the constitution of a professional order, 2) inclusion in another professional order, and 3) delegation of specific tasks from another regulated profession. Practical consideration of each model is a balancing act between public protection, and the resources required to seek legal recognition. Though legal recognition occurs at the provincial rather than federal level in Canada, we advocate for a pan-Canadian approach to develop strategies and resources to further provincial and territorial pursuit of legal recognition.

This work is part of the larger GenCOUNSEL study, funded through the LSARP Genome Canada competition with co-funding from: Canadian Institutes for Health Research, Genome BC, Genome Quebec, Provincial Health Services Authority, BC Children's Hospital Foundation and BC Women's Hospital Foundation.

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P23.015.C Implementing the use of genetic information in the electronic health record: a scoping review on the ethical and legal framework in the European context

*Paola Del Sette, Marco Salivetto, Camilla Tettamanti, Rosagemma Ciliberti, **Emilio Di Maria***

University of Genoa, Genova, Italy.

Genetic information includes family health history as well as data resulting from the analysis of biological samples. Data management is increasingly important to integrate relevant information into the electronic health record (EHR). In the European context, the GDPR has revised the data protection scheme to respond to the challenges of the digital technologies. Objective of the present study is to evaluate the existing knowledge about the implementation of genetic information in the EHR for clinical purposes, with focus on legal and ethical issues from the European perspective. A scoping review was designed according to the PRISMA-ScR extension to respond to the key question: What are the current recommendations and best practices on the implementation of genetic information into the EHR and its sharing among records of extended families?. The search strategy was piloted on PubMed. Out of 1608 records, the 500 best matching documents were screened. Grey literature was inspected as well. The major findings are briefly outlined. The definition of genetic data used in most official documents is restricted to genotype(s). A few articles addressed the broader term genetic information and family data. Most recent articles focussed on the use data collected by the mean of massive sequencing, and on possible abuse of such data. The sharing of genetic information within the family and with clinicians was not deeply explored so far; information inferred from family history was not addressed as well. The dialogue in the genomics community would need further investigation. *Partially funded by University of Genova FRA2017-2018.*

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P23.017.A New British Society for Genetic Medicine (BSGM) guidelines for ethical issues relating to genetic testing in childhood

***Alison E. Hall**[1], Rachel Hart[2], Angus Clarke[3]*

[1]PHG Foundation, Cambridge, United Kingdom, [2]West Midland Region Clinical Genomic Centre, Birmingham Women's and Children's Hospital, Birmingham, United Kingdom, [3]School of Medicine, Cardiff University, Cardiff, United Kingdom.

Introduction: The publication of the UK British Society for Genetic Medicine's (BSGM) consent and confidentiality guidance in July 2019 highlighted the need and desire for separate and updated guidelines in two areas, genetic testing in childhood, and prenatal genetic testing. The BSHG published guidance on the genetic testing of children in 2010, but this requires revision in light of challenges raised by new technological developments including whole genome sequencing (WGS), the mainstreaming of genomics and the wider integration of WGS and other genetic and genomic testing into routine health care via the NHS Genomic Medicine Service.

Methods: A multidisciplinary working group was formed in late 2019 under the auspices of the BSGM ethics and policy committee to update the 2010 BSHG genetic testing of children guidance. This group carried out a survey of BSGM members to solicit views on the continuing utility of the guidelines and the changes needed. A workshop was held in February 2020 to agree a draft outline, identify contents and the process for generating the draft.

Results: This presentation will highlight key aspects of the updated guidance. The guidance addresses relevant ethical and legal frameworks alongside case-based good practice for some of the ethical challenges that can be generated as a result of genetic and genomic testing. The intention is that this guidance is useable and readable for the expanding non genetic community undertaking genomic tests as well as for existing audiences. This guidance will be published in the summer of 2021.

A.E. Hall: None. **R. Hart:** None. **A. Clarke:** None.

P23.018.B Legal regulation of human genome editing in the Russian Federation

***Andrey A. Inyushkin**[1], Valentina D. Ruzanova[1], Elena M. Inyushkina[2], Olga A. Vedyasova[2], Margarita A. Zhuravleva[2], Alexey N. Inyushkin[2]*

[1]Department of Civil and Business Law, Samara National Research University, Samara, Russian Federation, [2]Department of Human and Animal Physiology, Samara National Research University, Samara, Russian Federation.

Introduction: The development of modern technologies has created new opportunities for carrying out genetic research and genome editing. On the other hand, scientists and potential users faced challenges, concerning civil and public regulations of genome editing and its clinical application. Here we discuss questions and legal issues related to the genome editing and its potential clinical uses in the Russian Federation.

Materials and Methods: Historical and comparative legal methods, methods of formal and dialectical logics, legal modelling were applied for the analysis of Russian law relating to the genome editing.

Results: Considering the Russian legal regulation, it should be noted that according to Art. 3 of the Federal Law of July 5, 1996 № 86-FL"On State Regulation in the Field of Genetic Engineering" the regulatory legal framework for genetic engineering, including genome editing, genome diagnostics and gene therapy, is made up by federal (not International) laws and laws of the subjects of the Russian Federation. This Law mainly regulates the issues of genetic engineering in agriculture and food production. Meanwhile, social relations associated with the diagnosis and editing of the human genome are rapidly developing and there is a vital need for solving of legal issues in this area. It is necessary to regulate in detail the issues of gene editing in relation to humans.

Conclusion: In our opinion, the normative legal regulation in this area needs to be expanded and brought into line with international legislation. The research was funded by RFBR according to the project № 18-29-14073.

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P23.019.C Laboratory perspectives on a template for reporting genomic sequencing results

Danya F. Vears[1,2,3], *Marjolein Kriek*[4], *Koen L. van Gassen*[5]

[1]*Centre for Biomedical Ethics and Law, Department of Public Health and Primary Care,KU Leuven, Leuven, Belgium,* [2]*Biomedical Ethics Research Group, Murdoch Children's Research Institute, Parkville, Australia,* [3]*Melbourne Law School, University of Melbourne, Carlton, Australia,* [4]*Leiden University Medical Centre, Leiden, Netherlands,* [5]*University Medical Center Utrecht, Utrecht, Netherlands.*

Existing research shows considerable variability in the information laboratories include in their genomic sequencing reports and their classification of variants. This suggests reporting guidelines are not being used consistently, which may have significant implications for patients.

To address this, we developed a report template based on the clinical reports we received from our previous study in which 41 laboratories analysed exome data from a virtual patient-parent trio. The template contents adhered to existing guidelines and was structured so that, even for very complex results, the most important information was on the first page. We invited these laboratories to complete a questionnaire to rate their satisfaction with each report component, overall length and layout.

Twenty-four laboratories from 13 countries assessed the template (RR = 58.5%). Support for inclusion of the Primary Findings section was high (96%) but fewer respondents agreed to include the Secondary Findings (71%) and Incidental Findings sections (58%). Rationales for excluding these sections often related to laws or laboratory-based practices which prevented reporting/searching for these types of findings or concerns about whether the patient had provided consent to receive them. Views on including the Clinical Management section were mixed with 75% of respondents agreeing to inclusion. Overall satisfaction with report length was high (79%), yet only 50% were satisfied with the level of detail provided.

These findings allowed us to refine the template, producing a tool which will assist laboratories to improve their reporting practices. This will increase report reproducibility and readability for clinicians, ultimately improving patient care.

D.F. Vears: None. **M. Kriek:** None. **K.L. van Gassen:** None.

P23.020.D New guidance from the British Society for Genetic Medicine about the ethical issues relating to prenatal genetic testing

Ruth Horn[1], *Alison Hall*[2], *Anneke Lucassen*[3], *BSGM Prenatal Genetic Testing Working Group*

[1]*Ethox Centre, University of Oxford, Oxford, United Kingdom,* [2]*PHG Foundation, University of Cambridge, Cambridge, United Kingdom,* [3]*Clinical Ethics and Law Unit, Faculty of Medicine, University of Southampton, Southampton, United Kingdom.*

Introduction: The publication of the UK British Society for Genetics Medicine (BSGM) consent and confidentiality guidance in 2019 highlighted the need and desire for separate and more detailed considerations of the ethical issues in two areas: genetic testing in childhood, and prenatal genetic testing. Substantial advances in the ability to detect genetic variation, at speed and low cost, together with the need to make these advances available to a much wider range of healthcare professionals - via the NHS Genomic Medicine Service - means that consideration of the ethical issues raised is timely.

Methods: A multidisciplinary working group was formed in late 2019 under the auspices of the BSGM ethics and policy committee to develop new guidance that focuses on areas highlighted by the BSGM consent and confidentiality guidance. This group carried out a survey of BSGM members to solicit views on the reach of existing guidelines and any changes that might be needed. A workshop was held in February 2020 to agree a draft outline, identify content and the process for generating the new guidance relating to pre-natal genetic diagnosis.

Results: This presentation will highlight key-aspects covered in the report. The document provides an overview of the types of prenatal genetic tests and the routes to their realisation with case-based illustrations of potential ethical and legal issues that arise in the patient-pathway. We explore the offer, delivery of testing, communication of results and subsequent management and hope that the report will facilitate ethical decision-making for both professionals and patients.

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P23.021.D The use of reflective diaries to explore the liminal space between clinical encounters in predictive Huntington's disease clinics

Lisa M. Ballard[1], *Shane Doheny*[2], *Angus Clarke*[2], *Anneke M. Lucassen*[1]

[1]*University of Southampton, Southampton, United Kingdom,* [2]*Cardiff University, Cardiff, United Kingdom.*

Introduction: What happens in a clinic appointment for a predictive Huntington's disease (HD) test has been documented in various ways. However, much less is known about the liminal space *between* those sessions. Our aim was to explore the following questions: 1) how does the decision to have a predictive test for Huntington's disease impact on patients' lives and 2) what does it feel like for patients to experience this process? This patient group was chosen because the pace of decision making does not usually allow for such detailed scrutiny of this liminal space.

Methods: We recruited 15 patients who were considering predictive testing for HD from four UK regional genetics services. Qualitative data was gathered from patients' reflective diaries to explore the impact of the deliberation process for a predictive HD

test and compared with data from clinical appointments. Data was analysed using thematic analysis, the voice approach and I-poems.

Results: We focused on topics identified in the reflective diaries that were not present in the clinic appointments. Analysis highlighted themes such as 'front and back-stage management', 'fear of stigmatisation', 'social responsibility', 'the absence of hope' and 'three imagined futures'. Voices and I-poems were used to illustrate these themes.

Conclusion: We used a participatory approach to answering the research questions, which was proportionate for the private nature of the diaries and the sometimes-emotive experiences they contained. It may be possible to develop these explorations of patient deliberation between clinical appointments to inform discussion within clinical appointments.ESRC Grant ES/R003092/1

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P23.022.B Lessons learned from incidental findings in clinical exome sequencing of 16,482 individuals

Vyne van der Schoot[1], *Lonneke Haer-Wigman*[2], *Ilse Feenstra*[2], *Femke Tammer*[2], *Anke J. M. Oerlemans*[2], *Martine P. A. van Koolwijk*[2], *Frans van Agt*[2], *Yvonne H. J. M. Arens*[1], *Han Brunner*[2], *Lissenka Vissers*[2], *Helger G. Ijntema*[2]

[1]*Maastricht University Medical Center, Maastricht, Netherlands,* [2]*Radboud university medical center, Nijmegen, Netherlands.*

Introduction: Incidental findings are unintentionally uncovered pathogenic variants predisposing to a disease unrelated to the clinical question. We report our experiences with IFs identified during 5 years of clinical exome sequencing.

Materials and methods: We evaluated IFs identified in 16,482 index patients receiving clinical exome sequencing, and compared these to 'ACMG59'-listed genes.

Results: IFs were identified in 0.58% (95/16,482) of index patients. The odds of IF differed significantly between analysis of restricted disease-gene panels (0.03%) and whole exome/Mendeliome analysis (1.03%). In 86 of 95 individuals, the IF was medically actionable. Sixty-one percent affected an 'ACMG59'-listed gene. The remaining 39% grouped into four categories: disorders similar to listed on 'ACMG59' (25%); disorders for which disease manifestation could be influenced (7%); IFs providing reproductive options (2%); and IFs with pharmacogenetic implications (5%).

Conclusion: The overall odds of IFs is low. IFs predisposing to medically actionable disorders affect a broader range of genes than 'ACMG59', advocating that pre-defined gene lists are too restrictive, and that IFs require ad hoc evaluation of medical actionability. Whereas both the identification and disclosure of IFs depend on local policy, the lessons learned provide essential insight into the nature and odds of IFs in clinical exome sequencing.

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P23.027.C Coping with healthcare needs among adolescents and young adults with Li-Fraumeni Syndrome (LFS): 'You're having to put up a fight to take care of your health'

Catherine Wilsnack[1], *Camella Rising*[1], *Pat Boyd*[1], *Alix Sleight*[2], *Sadie Hutson*[3], *Payal P. Khincha*[1], *Allison Werner-Lin*[4]

[1]*National Cancer Institute, Rockville, MD, USA,* [2]*Cedars-Sinai Medical Center, Department of Physical Medicine & Rehabilitation, Los Angeles, CA, USA,* [3]*3University of Tennessee Knoxville, College of Nursing, Knoxville, TN, USA,* [4]*University of Pennsylvania School of Social Policy and Practice, Philadelphia, PA, USA.*

Introduction: Li-Fraumeni Syndrome (LFS) is a rare hereditary cancer syndrome that confers nearly 100% lifetime cancer risk starting from birth. Early detection screening involves high-interval, burdensome, multimodal examinations. Adolescents and young adults (AYAs) with LFS may experience barriers to addressing unique healthcare needs in the US healthcare system, requiring compensatory strategies to cope with emotional and financial strain.

Materials and Methods: A multidisciplinary research team interviewed 38 AYAs with LFS (aged 16-38 years) who were enrolled in the National Cancer Institute's (NCI) LFS study (NCT01443468). Participants were predominantly female ($n = 26$) and white ($n = 31$) Interviews were recorded and transcribed, then analyzed using modified grounded theory.

Results: Participants described barriers related to accessing screening services or high quality LFS-related care, and inadequate financial resources for screening-related expenses. Most AYAs relied on the NCI to receive no-cost cancer screening and expressed worry about accessing screening after study termination. Participants in the control arm forewent cancer screening services to cope with cost burden. Financial planning supported coping with anxiety related to anticipated cancer costs. Participants demonstrated self-advocacy and served as educators to address dissatisfaction of healthcare providers rendering LFS care.

Conclusions: Participants discussed how their healthcare needs are disproportionate to coping capacity. Individuals are experiencing systems- and policy-level challenges yet employing individual-level coping strategies. Multi-level supportive interventions are lacking to ameliorate the burdensome physical, emotional, and financial challenges of AYAs with LFS. Results highlight the need for an integrated systems level solution to address barriers and optimize clinical care to promote adaptive coping strategies.

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P23.028.D Exploring the role of religion/spirituality on the lived experience of Muslim patients with Long QT syndrome: patients' and health professionals' perspectives

Khadijah Bakur[1,2,3], *Jumana Al-Aama*[4,3], *Zuhair Alhassnan*[5], *Helen Brooks*[6], *Tara Clancy*[7], *Waleed Manea*[8], *Saud Takroni*[9], *Fiona Ulph*[10]

[1]*Department of Genetic Medicine, King Abdulaziz University Hospital, Jeddah, Saudi Arabia,* [2]*School of Biological Science, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom,* [3]*Princess Al-Jawhara Centre of Excellence in Research of Hereditary Disorders, King AbdulAziz University, Jeddah, Saudi Arabia,* [4]*Department of Genetic Medicine, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia,* [5]*Department of Medical Genetics, King Faisal Specialist Hospital & Research Center, College of Medicine, Alfaisal University, Riyadh, Saudi Arabia,* [6]*Institute of Population Health Sciences, University of Liverpool, Liverpool, United Kingdom,* [7]*Manchester Centre for Genomic Medicine, St. Mary's Hospital, Manchester, Manchester, United Kingdom,* [8]*8 Heart center department, King Faisal Specialist Hospital & Research Centerr, Riyadh, Saudi Arabia,* [9]*Department of Medical Genetics, King Faisal Specialist Hospital & Research Center, Riyadh,*

Saudi Arabia, [10]Division of Psychology and Mental Health, Faculty of Biology, Medicine and Health, Manchester Academic Health Science Centre, University of Manchester, Manchester, United Kingdom.

Background: Genetic services are rapidly growing in many Islamic countries, leading to more diagnoses. Because Muslim patients integrate religion throughout their lives, its role in their coping and decision-making must be understood in the context of genetic counselling. This study explored the role of religion in the experiences of Saudi patients with long QT syndrome (LQTS), drawing on their perspectives and those of their health professionals.

Methods: The study employed semi-structured interviews to explore the role of Islam in patients' perceptions of the cause of diagnosis, coping strategies and decision-making. The participants were recruited from two Saudi Arabian cardio-genetic centres and investigated via interpretative phenomenological analysis. The study also used semi-structured interviews to explore health professionals' experiences regarding the role of patients' religion and spirituality in coping and decision-making. The health professionals were recruited from the same cardio-genetic centres, with the interviews thematically analysed.

Results: The interviews with 13 patients who had (or were carriers of) LQTS and 12 health professionals (clinical geneticists, genetic counsellors, cardiologists, molecular geneticists and patient coordinators) demonstrated that religion was significant in maintaining wellbeing in these patients. From both perspectives, the main factors influencing perceptions of the cause of diagnosis, coping and decision-making were the interpretations of religious beliefs and rulings and the availability and understanding of medical information.

Conclusion: Providing patients with clear medical information could alter their perceptions of the cause of diagnosis, which could contribute to better outcomes. Religious beliefs help reduce cognitive dissonance by casting wise decision-making as a religious duty.

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P23.030.B New British Society for Genetic Medicine (BSGM): Ethical issues relating to prenatal genetic testing

***Adeline Perrot**, Ruth Horn*

Ethox Centre, Oxford, United Kingdom.

Introduction: Non-invasive prenatal testing (NIPT) is a rapidly developing genomic technology that is constantly widening its scope and opening up new possibilities in reproductive medicine. Ten years after NIPT has been made commercially available, it is increasingly entering routine antenatal care as either a first- or second-tier test. In England, France and Germany, for example, NIPT has been made available free-of-charge as a second-tier test to women with a higher chance of common chromosomal anomalies. The clinical implementation of NIPT carries benefits but also raises important ethical questions. Our project analyses these questions within their specific contexts in England, France and Germany.

Methods: As part of a wider research project, which will involve qualitative methods, we conducted a document analysis to compare arguments about, and regulations governing NIPT in the three countries in: law and policy document; public reports; medical press; academic literature; and media.

Results: Despite the similarities between the three countries to offer NIPT as a second-tier screening tool, they exhibit differences with regard to their public discourses about prenatal genomics, screening policies, the risk-thresholds they use, professional regulations and laws. These differences have an impact on the way ethical issues emerge, and questions about the meaning of health, illness and disability, the scope of public health interventions, social inclusion and exclusion as well as reproductive choice are approached in each country.

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P23.031.C Healthcare professionals' perspectives and experiences with cases of nondisclosure of genetic information to relatives

***Amicia Phillips**[1], Danya F. Vears[1,2,3], Ine Van Hoyweghen[1], Pascal Borry[1]*

[1]University of Leuven, Leuven, Belgium, [2]Murdoch Children's Research Institute, Melbourne, Australia, [3]University of Melbourne, Melbourne, Australia.

Findings from genomic sequencing can have important implications for patients and relatives. Yet, when a patient does not consent to the disclosure of genetic information to relatives, it is unclear how healthcare professionals (HCPs) should balance their responsibilities towards patients and their family members and whether breaches in confidentiality are warranted. To address this issue, we interviewed 20 HCPs from Belgian genetics centers to explore how they addressed familial implications before and after testing, experiences with nondisclosure, and their views on various policies addressing nondisclosure. Participants identified various strategies for facilitating family communication but noted that constraints on time and resources hindered their ability to support patients with disclosure. Although cases of nondisclosure were uncommon, almost all HCPs reported having encountered such a case. HCPs felt tension between their duties to their patients and patients' relatives who could benefit from being informed of their genetic risk. There was substantial variation in the degree to which HCPs tried to persuade patients to communicate; several participants felt that by informing the patient of the importance of family communication, they had discharged their duty, while others took further measures to persuade patients. Participants felt that communication to relatives was primarily the responsibility of the patient but were hesitant to support policies that would formalize this responsibility. Participants also criticized policies that would permit or oblige HCPs to breach confidentiality, citing many practical limitations. Based on our findings, we recommend the development of clearer guidelines to help HCPs understand their duties and constraints.

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P23.032.D Parents' experiences with whole-exome sequencing in pediatric renal cancer

***Sebastian B. B. Bon**[1], Roel H. P. Wouters[1], Janna A. Hol[1], Marjolijn C. J. Jongmans[1,2], Marry M. van den Heuvel-Eibrink[1], Martha A. Grootenhuis[1]*

[1]Princess Máxima Center for Pediatric Oncology, Utrecht, Netherlands, [2]University Medical Center Utrecht, Utrecht, Netherlands.

Introduction: In pediatric renal cancer, germline whole-exome sequencing (WES) contributes to the identification of predisposing factors, facilitating surveillance and family counseling. Little is known about experiences and needs of families of childhood cancer patients regarding WES. Our qualitative interview study explores these experiences and needs in order to improve counseling and support.

Methods: Twenty-nine interviews were conducted with parents after they had been approached for a nationwide germline WES study in children with renal tumors, comprising a cancer panel analysis and optional exome-wide trio-analysis. The interviews were analyzed using an inductive thematic approach.

Results: Parents were generally positive about WES. Parents reported both individual and altruistic motives for participating. Altruistic motives such as contributing to science and helping future patients appeared more important after the child's treatment had been finished. Several families approached shortly after diagnosis reported feeling overwhelmed by the information. Parents often chose exome-wide (trio-)analysis over cancer panel analysis, although they faced significant difficulties distinguishing between these approaches. Families who received negative results felt relieved, but some worried about yet undiscovered factors or felt disappointed.

Conclusions: Families are positive about WES, however we identified several challenges pertaining to timing, consent and follow-up. We recommend approaching families during a relatively stable phase in their child's treatment trajectory. Separating consent for panel analysis and exome-wide analysis might help parents to make a deliberate decision. Attention should be payed to families who receive negative results.

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P23.033.A Definition of personalized medicine: links with familiarity and knowledge in genetics

Valentyn Fournier, *Loris Schiaratura*

University of Lille, Villeneuve-d'ascq, France.

Introduction: Personalized medicine (PM) is an important topic in public health. However, there is no consensus among its definitions. A recent study showed that familiarity with the medical field influences the definition of PM. Indeed, the most familiar people define it more technically with biomedical and genetic aspects, while the others only cite patient-centered aspects. In line with this study, our research proposes to replicate the effect of familiarity in a larger sample and to examine the role of genetic knowledge on the definition of PM.

Methods: 427 participants (205 in general population and 222 students, with different study domains) were recruited. They answered to an online questionnaire evaluating the definition of PM, attitudes towards pharmacogenomics and general beliefs towards medicines. Moreover, the general population participants answered to a genetic knowledge questionnaire.

Results: Independently of their study domain (scientific or not), participants characterize PM with both technical and patient-centered aspects. In the general population, the knowledge in genetics is significantly predictive of the definition of PM. The more genetically literate people are, the more they will define PM with genetic-related concepts.

Conclusions: The findings could lead to an improvement of patient care by taking into account knowledge in genetics. PM being a frequent tool in cancer care, it seems important for patients to understand what PM really implies in order to promote a better understanding and avoid oversized hopes.This research was funded by French National Cancer Institute, grant number 2018-164

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P23.034.B The views of health care providers on the scope of pre-implantation genetic testing: a systematic review

Maria Siermann[1], *Olga Tsuiko*[1], *Joris Vermeesch*[1], *Taneli Raivio*[2], *Pascal Borry*[1]

[1]*KU Leuven, Leuven, Belgium,* [2]*University of Helsinki, Helsinki, Finland.*

Introduction: Pre-implantation genetic testing (PGT) can be used to prevent passing on genetic conditions to future offspring or to improve reproductive success. Whereas in the past it was solely used for childhood-onset, lethal disorders, nowadays PGT is used for conditions with adult-onset, possible treatment and lower penetrance, in addition to non-medical traits such as sex selection. This can create dilemmas for health care providers around what should be the scope of PGT.

Materials and Methods: Four databases (Web of Science, PubMed, Embase, CINAHL) were systematically searched for qualitative empirical studies that investigated the perspectives of health care professionals on the scope of PGT. Multiple researchers independently performed a selection process and assessed included studies for methodological quality using the Critical Appraisal Skills Program.

Results: Twelve articles were included in our analysis. The main themes extracted were the providers' assessment of the 'seriousness' of genetic conditions, the tension between respecting patients' autonomy and provider's own viewpoint, and the dilemmas surrounding the expanding scope of PGT such as sex selection, non-medical traits, carrier embryos and comprehensive PGT.

Conclusions: In general, providers found the patients' viewpoint the most important in assessing appropriateness of PGT. However, providers differed in views of their role in limiting PGT: some preferred 'shared decision-making' between providers and patients, whereas others thought it should be the patients' choice. This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 813707 ('MATER': 'Innovative Training Network in Female Reproductive Care').

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P23.035.C Sociological aspects of preconception genetic screening for autosomal recessive diseases

Oksana V. Kopylova[1], *Karina Z. Revazyan*[1], *Alexey N. Meshkov*[1], *Alexandra I. Ershova*[1], *Anush M. Glechyan*[1], *Natalia A. Voinova*[2], *Alexander K. Volkov*[2], *Oxana M. Drapkina*[1]

[1]*National Medical Research Centre for Therapy and Preventive Medicine, Moscow, Russian Federation,* [2]*Bauman Moscow State Technical University, Moscow, Russian Federation.*

Introduction: The aim of the study was to identify attitudes towards preconception genetic screening (PGS) for common autosomal recessive diseases in family planning.

Materials and Methods: The study included 535 students and staff from one of Moscow Universities, age of 18-49 (female, 41%) and ≥18 (male). 83% were undergraduate; 61% were not married, 87% did not plan conception in the near future, 6% had relatives with hereditary diseases. Participants read a booklet on PGS of cystic fibrosis (CF), phenylketonuria (PKU), sensorineural hearing loss (SNHL), alpha-1-antitrypsin deficiency (A1AT) and filled out an anonymous questionnaire.

Results: The participants' answers were: PGS is useful regardless of family history (92%), it would help to avoid stress of having a sick baby (48%), PGS increases the chances of having a healthy baby (45%), carriage identification can change relationship between partners (46%) and attitude towards oneself (34%). 55% previously did not know about any of 4 diseases, the most known disease was SNHL (29%), the least known - A1AT (6%). Only 20% chose all correct statements on understanding of booklet information. 73% participants wanted to participate in PGS. The most common reason for consent was the desire to have complete information before the childbirth (62%); for refusal - absence of current relevance (70%). 15% chose the absence of relatives with hereditary diseases as a reason for refusal.

Conclusions: Most of the participants reacted positively to PGS. When introducing PGS into the health system, it is important to strengthen the knowledge of the population of PGS.

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P23.036.D Patient perspectives on making decisions in predictive genetic testing and in responses to fetal cardiac anomaly

*Shane Doheny[1], Lisa Ballard[2], Anneke M. Lucassen[2], **Angus J. Clarke**[1]*

[1]School of Medicine, Cardiff University, Cardiff, United Kingdom, [2]University of Southampton, Southampton, United Kingdom.

Introduction: The making of decisions by patients about genetic testing or about continuing a pregnancy in the face of genetic risk is often difficult. We focused on settings where decisions are personal choices not driven by medical treatment: testing for late onset disease with no treatment and decisions about continuing a pregnancy when the fetus has a cardiac anomaly. Patients were recruited as active co-researchers who reported their thoughts, feelings and family discussions about the "life world" within which their decisions are made.

Materials & Methods: We recruited 31 patients from predictive genetic testing clinics and seven patients from prenatal anomaly clinics. Data were collected from clinics, diaries and interviews. We mapped the content of all data sources and selected key parts of the data for discourse analysis, attending to what was (not) said and considering questions of influence and persuasion.

Results: Diaries can provide rich information about the life world within which patients make decisions, giving insight into factors that may not be discussed in clinic. Differences of perspective within a family were found to be important factors in the making of decisions. Although there were few recordings of family discussions, these can be especially rich.

Conclusions: Asking patients to keep a diary allows access to difficult and sensitive aspects of their decision making relevant to genetic counselling. Our approach provides novel insights of use in training practitioners. We suggest ways to develop these methods for research and adapt them for use in genetic counselling. ESRC Grant Ref: ES/R003092/1

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P23.037.A Uptake of Fetal Aneuploidy Screening After the Introduction of the Non-Invasive Prenatal Test: a National Population-based Register Study

***Karuna R. M. van der Meij**[1], Maurike de Groot- van Mooren[1], Caroline Kooij[1], Ellen W. S. Carbo[2], Mijntje J. Pieters[3,4], Wendy Rodenburg[2], Robert-Jan H. Galjaard[5], Erik A. Sistermans[1], Martina C. Cornel[1], Lidewij Henneman[1], The Dutch NIPT Consortium*

[1]Amsterdam UMC, Amsterdam, Netherlands, [2]National Institute for Public Health and the Environment, Bilthoven, Netherlands, [3]Maastricht University Medical Center, Maastricht, Netherlands, [4]Foundation Prenatal Screening Southeast region of the Netherlands, Maastricht, Netherlands, [5]Erasmus Medical Center, Rotterdam, Netherlands.

Introduction: Countries are exploring ways to integrate the Non-Invasive Prenatal Test (NIPT) in their prenatal screening programs, either as a first- or second-tier test. This study describes how the uptake of fetal aneuploidy screening changed after the introduction of NIPT as a second-tier and as a first-tier test within the Netherlands.

Methods: A population-based register study, recording uptake of fetal aneuploidy screening in the Netherlands. Data from all pregnant women choosing to have the First-trimester Combined Test (FCT) or first-tier NIPT between January 2007 and March 2019 were retrospectively collected using national registration systems. For 2018, postal codes were used to compare NIPT uptake between socioeconomically disadvantaged neighborhoods and other neighborhoods.

Results: After the introduction of NIPT as a first-tier test for all women in April 2017 (TRIDENT-2 study), FCT uptake declined significantly from 35.8% in 2016 to 2.6% in 2018 ($p < 0.0001$). NIPT uptake increased to 43.4% in 2018. Total uptake (FCT and NIPT) between 2007 and 2018 increased significantly from 14.8% to 45.9% ($p < 0.0001$). However, total uptake stabilized at 46% for both years of TRIDENT-2. NIPT uptake in socioeconomically disadvantaged neighbourhoods was 20.3% whereas NIPT uptake in other neighbourhoods was 47.6% ($p < 0.001$).

Conclusions: An increase in total fetal aneuploidy screening uptake up to 45.9% was observed after the introduction of NIPT. Uptake appears to have stabilized within a year after introducing first-tier NIPT. The results indicate potential unequal access to NIPT, which has both ethical and policy implications. **Funding:** ZonMw Netherlands grant no. 543002001

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P23.038.B Ethical aspects of genomic sequencing in neonatology

Elena Grebenshchikova

Institute of Scientific Information for Social Sciences; Pirogov Russian National Research Medical University, Moscow, Russian Federation.

Introduction: Advances in genetic technologies are gradually making genetic screening cheaper and more accessible. The prospect of a "genomic revolution" as declared by Matt Hancock, the UK's health secretary, announced a plan to sequence the genome of all babies born in a NHS hospital, raises many ethical questions in a broader bioethical context.

Materials and methods: basic principles of bioethics (do no harm, beneficence, respect for autonomy and justice) have been used to analyze ethical issues.

Results: Based on the principle of do no harm, it is necessary to take into account the damage that may arise for both the child and the parents. For example, there is no consensus on the need to inform about late manifestation diseases. The principle of beneficence brings into question our understanding of good. For example, the idea of the good can vary significantly in different sociocultural contexts. The principle of respect for individual autonomy presupposes self-determination of the individual. However, instead of the newborn, the decision is made by the parents. "Choose instead of" can be a tricky question given the incidents of "wrongful life suits". The principle of justice raises many ethical issues related to access, allocation of scarce health care resources, etc.

Conclusion: The analysis of ethical issues of genomic sequencing based on the basic principles of bioethics has considerable theoretical potential. The research was supported by the grant of the Russian Scientific Foundation, project № 19-18-00422.

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P23.039.C Product liability landscape in the EU: the case of DTC genetic testing within the in vitro diagnostic medical devices regulation (IVDR) framework

Klea Vyshka[1,2,3], *Alain Verloes*[1,4,3]

[1]*Dept. of Genetics, Assistance Publique-Hôpitaux de Paris - Université de Paris, Paris, France,* [2]*CERCRID, UMR 5137 "Centre de Recherches Critiques en Droit", Université de Lyon, Saint-Etienne/Lyon, France,* [3]*European Reference Network For Intellectual Disability, Telehealth And Congenital Anomalies (ERN-ITHACA), Paris, France,* [4]*INSERM UMR 1141 "NeuroDiderot", Hôpital R DEBRE, Paris, France.*

Introduction: The EU law landscape on in vitro diagnostic medical devices, applicable to direct-to-consumer (DTC) genetic tests, has been enriched in 2017 with a new regulation (IVDR). Despite the ever-growing attention the EU legislator dedicates to safety and marketing of medical devices and pharmaceutical products, the liability regime for potentially defective products remains the same as described in the maximum harmonisation directive on product liability (PLD), in force since 1985.

Methods: DTC genetic testing enterprises, after an already established practice in the USA, are progressively interested in selling their products in the EU single market, one of the largest and richest in the world, endowed with educated and empowered patient-consumers. Through an analysis of the relevant EU legislation and applicable national laws, this contribution aims to answer the question whether the EU product liability directive is suited to litigations on health-related products in general and DTC tests in particular.

Results: Even though the PLD may have raised numerous questions with regard to its adaptability with advanced technological devices, its system of no-fault, strict liability of the manufacturers remains effective.

Conclusion: The PLD broad definition of manufacturer, combined with the IVDR obligation for non-EU manufacturers to designate a sole authorised representative in the territory, provides satisfactory protection for the consumers. Thus, in every litigation the EU manufacturer or its representative is identifiable. In this reading, these instruments are complementary to each-other and can establish as such a special product liability regime applicable to IVDR products. This research is supported by ERN-ITHACA.

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P23.040.D The psychosocial experience of families with hereditary amyloid transthyretin amyloidosis with polyneuropathy: a mixed-methods systematic review

José D. Pereira[1,2], *Andreia Santos*[3], *Eugenia Cisneros*[4,5], *Intissar Anan*[6], *Marina S. Lemos*[7,8], *Milena Paneque*[1,2]

[1]*CGPP - Centro de Genética Preditiva e Preventiva and UnIGENe, IBMC - Instituto de Biologia Molecular e Celular, i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal,* [2]*Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal,* [3]*Póvoa de Varzim, Porto, Portugal,* [4]*Servicio de Medicina Interna, Hospital Universitario Son Llàtzer, Palma de Mallorca, Spain,* [5]*Institut d'Investigació Sanitària Illes Balears, Hospital Universitario Son Espases, Palma de Mallorca, Spain,* [6]*Institutionen för folkhälsa och klinisk medicin, Umeå universitet, Umeå, Sweden,* [7]*Faculdade de Psicologia e de Ciências da Educação, Universidade do Porto, Porto, Portugal,* [8]*Centro de Psicologia, Universidade do Porto, Porto, Portugal.*

Introduction: Hereditary amyloid transthyretin amyloidosis with polyneuropathy, besides its chronicity and devastating progression, poses a strong psychological impact on patients and their relatives. A systematic review about the psychosocial experience of these families may help health professionals to provide them with the best possible care.

Methods: Manuscripts published between January 1992 and December 2019 were searched using 16 databases. The work includes a methodological quality assessment of the selected studies, a postsynthesis sensitivity analysis, and an overall assessment of the thematic synthesis.

Results: Of 7,394 manuscripts identified through database searching, 220 were reviewed in full text and 57 met the eligibility criteria. Although scarce, the data on psychosocial issues present in those studies suggest that the disorder and its life implications provoke a significant psychosocial burden for these families. The decision about up taking presymptomatic testing and seeking reproductive options are other sources of emotional impact.

Conclusions: Psychosocial experience of these families is not enough studied. Although literature has described their life paths, further research on other key disease variables (including the psychosocial experience based on timing of clinical onset in the life cycle and effects of the most recent treatment interventions) can fill gaps and optimize health care services that support the families with the condition. José D. Pereira has a Ph.D. grant (SFRH/BD/138012/2018), financed by the Fundação para a Ciência e a Tecnologia through the Human Capital Operational Programme, co-participated by the European Social Fund and by national funds from the Ministério da Ciência, Tecnologia e Ensino Superior.

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P23.041.A How to accomplish public dialogue about human germline editing? The example of the Dutch DNA dialogues

Sam Riedijk[1], *Diewertje Houtman*[1], *Marcia van Woensel*[2], *Eef Grob*[3], *Eline Gorter*[4], *Jacqueline Pot*[3], *Petra Verhoef*[5]

[1]*Erasmus Medical Center, Rotterdam, Netherlands,* [2]*Nemo Kennislink, Amsterdam, Netherlands,* [3]*Erfocentrum, Amersfoort, Netherlands,* [4]*NPV Zorg voor het leven, Veenendaal, Netherlands,* [5]*Rathenau Institute, Den Haag, Netherlands.*

Introduction: Public dialogue about human germline editing (HGGE) is globally endorsed, but not many initiatives have been deployed. It requires expertise that most genomics professionals

do not possess. Therefore, we assembled a multidisciplinary consortium consisting of technology assessment-, science communication- and genomics experts and created an innovative dialogue format to raise awareness and empower opinion formation among a broad and diverse public.

Methods: Most professionals intuitively resort to explaining HGGE technology when attempting to engage the public. This deficit model approach to science communication is a sticky concept, although demonstrably outdated. Instead of sending information, we created three short, adaptive animations of a future society in which HGGE has (not) been embedded. Employing a dialogue model, as opposed to debate, the moderator invited participants to exchange perspectives and assemble thoughts, feelings, doubts and questions. Experts were not given a stage but were seated among participants, and instructed to contribute only to stimulate the conversation, not to direct it. To attract various participants we employed a targeted media strategy and creatively involved our networks.

Results: This resulted in 27 dialogues in one year with a broad variety of publics, numerous accounts of dialogues in (social) media and a summarizing report for our Ministry of Health, Welfare and Sports.

Discussion: Our interdisciplinary approach has led to the creation of a sustainable dialogue format that allowed to stimulate opinion formation as well as assembly of public opinions on HGGE. Overall, acceptance of HGGE for severe hereditary disease was high, but highly conditional.

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P23.042.B Assessing the outcomes of public engagement in genomics: the case of the Dutch DNA-dialogues

Diewertje Houtman[1], *Boy Vijlbrief*[1], *Marike Polak*[2], *Jacqueline Pot*[3], *Petra Verhoef*[4], *Martina Cornel*[5], *Sam Riedijk*[1]

[1]*Erasmus Medical Center, Rotterdam, Netherlands,* [2]*Erasmus University Rotterdam, Rotterdam, Netherlands,* [3]*Erfocentrum, Amersfoort, Netherlands,* [4]*Rathenau Instituut, The Hague, Netherlands,* [5]*Amsterdam UMC, Vrije Universiteit Amsterdam, Amsterdam, Netherlands.*

Introduction: This study reports on the acceptance of human germline gene editing (HGGE) among visitors and non-visitors of 25 public dialogues in The Netherlands. Public perspectives on HGGE have often been called for and are deemed necessary for democratic decision-making about potential future applications and current proceedings. In addition, comparing acceptance before and after dialogue signals the potential impact of participation.

Methods: Aiming to generate data on national and dialogue levels, questionnaires on opinions were filled out (1) before and after dialogue (T0 & T1; $n = 33$) by a subgroup of visitors, (2) before or after dialogue by subgroups of people who had either signed up for dialogue (T0; $n = 283$) or who completed the questionnaire after they entered in dialogue (T1; $n = 110$), and (3) in August 2019 (T0; $n = 1172$) and April 2020 (T1; $n = 1209$) by independent samples from the Dutch population. Questions included acceptance of different applications of HGGE, which we compared between groups using t-tests.

Results: Table 1 shows the different subgroups and their acceptance of different applications. Comparisons of T0 and T1 measures show a difference in independent dialogue responses, with higher acceptance of HGGE to prevent a severe muscular disease after dialogue.

Conclusions: The results might indicate that for some visitors, dialogue increases the acceptance of HGGE for severe heritable diseases, possibly because participants exchange perspectives with people who experience such diseases.

Table 1 *Changes in acceptance of applications mean scores, T0 to T1*

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P23.043.C Rare Diseases in Georgia Results of a Two-Year Study

Tinatin Tkemaladze[1,2,3,4], *Elene Abzianidze*[1], *Mariam Ghughunishvili*[1,2], *Irakli Rtskhiladze*[3], *Tamar Ramishvili*[3], *Volha Skrahina*[4], *Arndt Rolfs*[4,5,6]

[1]*Tbilisi State Medical University, Tbilisi, Georgia,* [2]*Givi Zhvania Pediatric Academic Clinic, Tbilisi, Georgia,* [3]*Medical Center Mrcheveli, Tbilisi, Georgia,* [4]*Centogene GmBH, Rostock, Germany,* [5]*University Rostock, Rostock, Germany,* [6]*Arcensus GmbH, Rostock, Germany.*

Introduction: Diagnosing rare diseases (RD) is challenging for doctors in many countries, especially in underdeveloped low-income countries like Georgia. Because of the rarity of these conditions and the high price of genetic investigations awareness of RDs is limited and many patients remain undiagnosed.

Aim: Our aim was to increase awareness of RDs among the healthcare professionals and the public, and enable diagnosis of RD patients.

Methods: From 2018 we initiated Biomarker Clinical Study with Centogene for various IEMs, which enabled us to offer free of charge testing for suspected patients. We carried out several workshops "Application of Artificial Intelligence in the Diagnosis of RDs" together with FDNA for doctors and medical students. We also initiated "Rare Disease" column in one of Georgia's largest social media medical groups, where each week information on a particular RD is uploaded into Georgian language. Additionally, we started collaboration with Unique and information on various RDs will now be available in Georgian language as well.

Results: As a result of the above-mentioned activities awareness of RD has significantly increased. In 2018-2020 about 2000 individuals received genetic testing. WES had up to 60% diagnostic yield in children with neurodevelopmental disorders, with 4 missed PKU cases identified through untargeted WES. Several patients' organizations were formed and more are underway. Our goal in future is to further promote education of doctors and students to recognize RD, support formation of parent's organizations and patient advocacy groups, negotiate with government to support RD patients and facilitate improvement national NBS program.

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P23.044.D Standardised tool for measurement of rare genetic disease costs: development, contextualisation, translation, and validation of the Client Service Receipt Inventory

Claudia C. Y. Chung[1], *Jasmine L. F. Fung*[1], *Adrian C. Y. Lui*[1], *Marcus C. Y. Chan*[1], *Yvette N. C. Ng*[1], *Wilfred H. S. Wong*[1], *So Lun Lee*[2,3], *Martin Knapp*[4], ***Brian H. Y. Chung***[1,3]

[1]*University of Hong Kong, Hong Kong, Hong Kong,* [2]*Queen Mary Hospital, Hong Kong, Hong Kong,* [3]*The Duchess of Kent Children's Hospital, Hong Kong, Hong Kong,* [4]*London School of Economics and Political Science, London, United Kingdom.*

Background: The measurement of costs is challenging, but is fundamental in healthcare decision-making. Standardised methods have not been developed in the rare disease (RD) population. This study aims to develop, contextualise, translate, and validate the Client Service Receipt Inventory for RDs (CSRI-Ra).

Methods: Two focus group meetings were conducted, involving RD patients, carers, and healthcare and social-care professionals from Hong Kong to understand the needs of RD population and the local healthcare and social-care systems. Data were analysed using thematic analysis. The CSRI-Ra was developed. Forward and backward translations were performed. Interviews with RD patients, carers, and healthcare professionals were conducted to achieve face validity and semantic equivalence. Intra-class correlation coefficient (ICC) was used to estimate the inter-rater reliability between English-Chinese translations, and between CSRI-Ra and electronic patient record (ePR).

Results: Emerging themes identified from focus group meetings were grouped into five sections in the CSRI-Ra: background, household and carer support, healthcare service and resource utilisation, community support, and education and employment. Excellent reliability was achieved between English-Chinese translations (ICC 0.91; 95% CI 0.89-0.92). Moderate-to-good reliability was achieved between CSRI-Ra and ePR (ICC 0.69; 95% CI 0.56-0.78). Following rounds of revision in the development, contextualisation, translation, and validation stages, the CSRI-Ra is ready for use in empirical research.

Conclusion: The CSRI-Ra provides a sufficiently standardised yet adaptable method for collecting socio-economic data related to RDs. Adaptation of the tool to other populations would facilitate international research.

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P23.045.A Responsible research and innovation in genetics: the challenge of preventive clinical genetics

***Boy Vijlbrief**, Diewertje Houtman, Sam Riedijk*

Erasmus University Medical Center, Rotterdam, Netherlands.

Developments in clinical genetics are increasingly moving towards the possibility of preventive clinical genetics (PCG), opposed to current indication-based paradigms. While expected to benefit many, this technology is also expected to disrupt common practice and raise difficult questions regarding utility, feasibility, and permissibility. Central to these concerns is the question: what are key characteristics of responsible research and innovation (RRI) of PCG? Data triangulation was performed between 8 semi-structured interviews with clinical genetics experts on PCG, analysis of ESHG 2020 presentations, and multidisciplinary meetings with genetics experts on ethical issues of PCG, to collect and compare expert perspectives on PCG and RRI. Results are marked by wide discrepancies, such as in motivation (meeting DTC demand in a hospital setting vs. establishing biobanks), envisioned target groups (PCG as universal population screening vs. additional diagnostic tool for hereditary cancer), uses (pharmacogenetics vs. severe monogenic actionable mutations), and evaluation criteria (health expenditure vs. self-reported empowerment of patients). Appraisal of discrepancies by experts is typified by shock at developments and possibilities, and an inability to establish a common language between disciplines and research groups. Notably, there seems to be little knowledge concerning target group needs besides extrapolation from DTC demand. Results show RRI of PCG requires development of common language between experts, and alignment between their motivations, practices, evaluation criteria etc. to cooperatively assess and design responsible PCG practices. To ensure alignment between PCG benefits and target group needs, strong dialogical engagement of possible target groups is required, to be facilitated by PCG experts.

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P23.046.B Scarcity of available information resources for patients and clinicians after a diagnosis of ultra-rare diseases: retrospective on a cohort of 626 individuals with congenital abnormalities and/or intellectual disability

***Paul Rollier**[1,2], Sandrine de Montgolfier[3,4], Christèle Dubourg[1,2], Wilfrid Carré[1,2], Véronique David[1,2], Marie-Dominique Galibert[1], Chloé Quelin[1], Mélanie Fradin[1], Alinoë Lavillaureix[1,2], Godelieve Morel[1], Laurent Pasquier[1,5], Sylvie Odent[1,2], Nolwenn Jean-Marçais[1,5]*

[1]Rennes University Hospital, Rennes, France, [2]Institute Genetics & Development of Rennes (IGDR CNRS UMR 6290), Rennes, France, [3]Interdisciplinary Institute of Social Issues (IRIS UMR8156 - U997), Aubervilliers, France, [4]Institut national supérieur du professorat et de l'éducation (INSPE), Creteil, France, [5]CIC-P Inserm 1414, Rennes, France.

Introduction: With development of high-throughput sequencing, diagnostic yield in congenital malformations (CM) and/or intellectual disability (ID) has increased rapidly as has the share of ultra-rare diseases (URD: prevalence<1/50 000) diagnosed. Among these URD, ratio of recently described diseases with little hindsight on clinical course and with small descriptive cohorts appears to be significant.

Materials and Methods: We used exome data of 626 individuals with CM and/or ID (without specific clinical orientation) sequenced between 2017 and 2020 at the Molecular Genetics Laboratory of Rennes University Hospital (France). DNA samples were analyzed by exome sequencing (Agilent kit) on Illumina platforms. Among these diagnoses, we researched URD and information resources available to patients and clinicians.

Results: Detection of ACMG (likely) pathogenic variants in 142 different genes allowed us to make a diagnosis in 208 cases (33.23%). 83.7% of these diseases, whose prevalence is available via Orphanet database, are URD. For these diagnoses, mostly of recent descriptions (55.3% after 2011, 23.4% after 2016), limited information (patient association, web resources) is available to patients and clinicians.

Conclusions: All these data underscore the significant proportion of URD diagnosed in CM/ID and the limited information available to patients and their families on their evolution and management. This can lead to a feeling of isolaton, which requires special medical follow-up procedures accompanied by regular updating of knowledge. In view of the increase in diagnosis of URD, it seems fundamental to establish them as a research subject and to study the ethical issues surrounding the announcement and accompaniment of such diagnostic results.

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P23.047.C Motives for withdrawal of participation in biobanking and participants' willingness to allow linkages of their data

Reinder Broekstra[1,2], *Judith L. Aris-Meijer*[1], *Els L. M. Maeckelberghe*[1], *Ronald P. Stolk*[1], *Sabine Otten*[1]

[1]*University of Groningen, University Medical Center Groningen, Groningen, Netherlands,* [2]*University of Southampton, Southampton General Hospital, Southampton, United Kingdom.*

Biobanks and data-sharing projects seek to optimize public participation rates while simultaneously attempting to increase the amount of data donated per participant. All these efforts should foster development of learning health systems and personalized medicine, especially when data linkage is permitted by participants. We investigated individuals' motives for participating in such projects and potential reasons for their withdrawal from participation in a population-based biobank. We also analysed how these motives were related to various characteristics of the participants and their intention to permit data linkage to their personal data for research. These questions were explored using sample of a participants in the Dutch Lifelines biobank (n = 2,615). Our results indicated that motives for participation and withdrawal were premised on benefits or harm to society and to the individuals themselves. Although general values and trust both played key roles in participation, potential withdrawal, and willingness to permit data linkage, they were differentially associated with motives for participation and withdrawal. These findings support and nuance previous findings by highlighting the distinctiveness and complexity of decision making regarding participation or withdrawal from data donation. Moreover, we suggest some new directions for improving recruitment, retention and safeguarding strategies in biobanking. Moreover, our data provide initial evidence regarding how factors may relate with the probability that individuals will agree to data linkages, when controlling for their unique effects. Future research should further investigate how perceptions of societal and individual harm and benefits may influence decision making on withdrawal of participation.

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P24 GWAS

P24.001.D The *COL1A1* gene variants and sports-related musculoskeletal soft-tissue injuries in Lithuanian athletes

Valentina Gineviciene, *Kamile Venckute, Algirdas Utkus*

Faculty of Medicine, Vilnius University, Vilnius, Lithuania.

Introduction: Single nucleotide polymorphisms (SNPs, rs1107946 and rs1800012) within the *COL1A1* gene, an important regulator of fibril assembly in tendons, have previously been associated with sports-related musculoskeletal soft-tissue (MSST) injuries. The aim of this case-control study was to examine the association of these *COL1A1* SNPs with MSST injuries in Lithuanian elite athletes group.

Materials and Methods: A total of 62 athletes, with a history of MSST injuries (shoulder dislocations, anterior cruciate ligament & meniscus tears; Achilles tendon ruptures & tendinopathy) and 123 control athletes (CON) without any reported history of MSST injuries were genotyped for the *COL1A1* rs1107946 (G>T) and rs1800012 (G>T) variants (using Real-Time PCR *Taqman* genotyping assays).

Results: Genotype distributions for both SNPs among cases and controls conformed to Hardy-Weinberg equilibrium (p>0.05). The *COL1A1* rs1107946 TT genotype was significantly over-represented in the MSST injuries (case) group (11%) when compared to the CON group (3%, p = 0.042). The odds ratio (OR) of case athletes harboring rs1107946 TT genotype compared to CON was 5.09 (95%CI:1.27-20.43, p = 0.022). The distribution of genotype frequencies of *COL1A1* rs1800012 variant in the cases significantly differed from the CON (GG/GT/TT: 44/32/24% vs 89/10/1%; p < 0.0001). The frequency of the rs1800012 T allele was significantly higher in cases (40%) compared to that in the CON (6%, p < 0.0001). The OR of athletes with MSST injuries harboring *COL1A1* rs1800012 TT genotype compared to CON was 38.9 (95%CI:5-303, p = 0.0005).

Conclusions: This study revealed an association among the SNPs *COL1A1* rs1107946 (TT genotype) and rs1800012 (TT genotype) and MSST injuries in Lithuanian athletes.

V. Gineviciene: None. **K. Venckute:** None. **A. Utkus:** None.

P24.002.A Genome-wide association meta-analysis identifies 12 novel loci for age-related hearing loss

Natalia Trpchevska[1], *Neelke B. C. Oosterloo*[2], *Maxim B. Freydin*[3], *Linda Broer*[2], *Paul A. Nagtegaal*[2], *Andre Goedegebure*[2], *Christopher R. Cederroth*[1,4], *Frances M. K. Williams*[5], *EARGEN consortium*

[1]*Karolinska Institutet, Stockholm, Sweden,* [2]*Erasmus Medical Center, Rotterdam, Netherlands,* [3]*King's College, London, United Kingdom,* [4]*Nottingham University, Nottingham, United Kingdom,* [5]*King's College, London, United Kingdom.*

Introduction: Age-related hearing loss (ARHL) is a complex polygenic disorder, but relatively few genomic loci have been identified so far. The EARGEN consortium was initiated to systematically discover new loci underlying HL and conducted the largest meta-GWAS to date.

Methods and Materials: The sample comprises 14 independent cohorts including the UKBiobank and FinnGen. Summary GWAS statistics were obtained for each cohort. The phenotype was based on self-reported HL and ICD9/10 diagnosis for sensorineural HL. EasyQC was applied in order to unify and harmonize datasets. Meta-analysis was performed with METAL software. Post-GWAS analyses were carried out using FUMA and VEGAS2 software.

Results: Our total sample comprised 724,756 Caucasian individuals (148,471 cases and 576,595 controls). We found 46 independent loci, of which 12 were novel. Many previously identified loci were confirmed, including loci harboring rare variants for non-syndromic HL (e.g. EYA4 and TRIOBP), as well as variants involved in hearing function such as CTBP2, a component of the presynaptic machinery, and CLRN2, which maintains stereocilia integrity and function. Five new genes (SPTBN1, CCDC87, KCTD10, MYO1H, MMAB) were prioritized for functional follow-up. Pathway analysis confirmed the involvement of sensory perception of sound, actin-binding, and filament polymerization in ARHL.

Conclusion: A GWAS meta-analysis of 724,756 individuals identifies 12 novel loci associated with ARHL while confirming previously reported genes in either mice or humans.

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P24.004.C Genome-wide association study of sex effect on asthma susceptibility in African-admixed populations

Antonio Espuela-Ortiz[1], *Esther Herrera-Luis*[1], *Fabian Lorenzo-Diaz*[1,2], *Michael A. Lenoir*[3], *Jose R. Rodriguez-Santana*[4], *Esteban G. Burchard*[*5,6], *Maria Pino-Yanes* [*1,7,8]

[1]*Genomics and Health Group, Department of Biochemistry, Microbiology, Cell Biology and Genetics, Universidad de La Laguna, San*

Cristóbal de La Laguna, Tenerife, Spain, [2]Instituto Universitario de Enfermedades Tropicales y Salud Pública de Canarias (IUETSPC), Universidad de La Laguna, San Cristóbal de La Laguna, Tenerife, Spain, [3]Bay Area Pediatrics, Oakland, CA, USA, [4]Centro de Neumología Pediátrica, San Juan, PR, USA, [5]Department of Bioengineering and Therapeutic Sciences, University of California San Francisco, San Francisco, CA, USA, [6]Department of Medicine, University of California San Francisco, San Francisco, CA, USA, [7]Instituto de Tecnologías Biomédicas (ITB), Universidad de La Laguna, San Cristobal de La Laguna, Tenerife, Spain, [8]CIBER de Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Spain.

Introduction: Sexual dimorphism in humans is presented in a variety of forms and affects both physical traits and disease-related phenotypes. One of the diseases affected by sexual dimorphism is asthma, a respiratory disease characterized by dyspnoea, cough, chest tightness, and wheezing. Asthma prevalence changes throughout the lifespan and differs by sex, being more prevalent in males during childhood and in women after puberty. Here we aimed to study the influence of sex on the genetic susceptibility to asthma in African-admixed populations.

Materials and methods: A total of 4,291 Hispanic/Latino and 1,657 African American subjects were analysed. Genetic data imputed using 1000 Genomes Project was used to perform genome-wide association studies (GWAS) following two different approaches, sex-interaction and sex-stratified analyses, and results obtained from each cohort were meta-analysed.

Results: In the interaction GWAS, no variants were associated at a genome-wide significant level, but suggestive associations at 2q22.1 were detected ($p\text{-value}_{interaction}<10^{-6}$). In the stratified GWAS, these variants had a protective effect in females and risk in males. On the other hand, stratified GWAS highlighted that 17q12-21 asthma locus has a contribution in both sexes ($p\text{-value}_{female} < 4.47x10^{-8}$; $p\text{-value}_{male} < 9.5x10^{-3}$), while the genomic region 4p15.31, involved in height determination and spermatogenesis, was only significant in males ($p < 4.3x10^{-8}$).

Conclusion: Despite having a common genetic basis, our results suggest that asthma pathogenesis acts through different mechanisms in males and females. Funding: Funded by SAF2017-83417R MINECO/AEI/FEDER, UE, and a MICIU/ULL fellowship to AE-O.

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P24.005.D Harnessing tissue-specific genetic variation to dissect the causal pathways between adiposity and complex disease

***Genevieve Leyden**, Chin Yang Shapland, George Davey Smith, Michael Greenwood, David Murphy, Tom G. Richardson*

University of Bristol, Bristol, United Kingdom.

Introduction: Body-mass index (BMI) is a risk factor for complex disease known to be influenced by genes acting via both metabolic pathways and appetite regulation. In this study, we aim to gain insight on the disease impact of BMI associated genetic variants mediated by their expression in different tissues.

Methods: First, we harnessed meta-analyzed gene expression datasets derived from adipose ($n = 1157$) and brain ($n = 1194$) tissues to provide insight into the underlying mechanisms at 915 genome-wide loci for BMI ($r^2<0.01$, $p < 5x10^{-8}$). Next, we developed a novel extension of multivariable Mendelian randomization (MVMR) to separate the effects of adipose- and brain-regulated BMI on 5 disease outcomes.

Results: 86 and 140 loci provided evidence of genetic colocalization with adipose and brain-derived gene expression respectively, suggesting that the genetic variants which influence BMI at these loci also influence proximal gene expression in these tissues. Overall, we found that the adipose colocalized variants

		Mean score			Difference of means paired t-test *p*-value	Difference of means independent samples t-test *p*-value	
Acceptance ofapplication for:	**Range**	**Paired dialogue responses**	**Independent dialogue responses**	**Independent population samples**	**Paired dialogue responses T0 vs. T1.**	**Independent dialogue responses T0 vs. T1**	**Independent population samples T0 vs. T1**
Severe muscular disease	1-5				0.801	0.025*	0.472
Pre-measurement (T0)		4.45	3.60	3.80			
Post-measurement (T1)		4.42	3.92	3.83			
Infectious disease	1-5				1.000	0.973	0.385
Pre-measurement (T0)		2.90	2.51	2.97			
Post-measurement (T1)		2.90	2.50	3.01			
Intelligence	1-5				1.000	0.805	0.912
Pre-measurement (T0)		1.52	1.56	1.91			
Post-measurement (T1)		1.52	1.54	1.90			

were enriched for waist:hip ratio (adjusted for BMI) (P = 0.0003) suggesting that they are typically involved in fat distribution. The MVMR reveals that tissue partitioned variants have distinct biological contributions, with brain-regulated BMI driving the effect on outcomes such as coronary artery disease P = 0.009; OR = 1.52; 95%-CI:1.11-2.08) and type-2 diabetes (P = 0.0007; OR:2.66; 95%-CI:1.5-4.7), whereas adipose-regulated BMI was responsible for the effect on osteoarthritis risk (P = 0.01; OR:1.86; 95%-CI:1.16-2.98).

Conclusion: This study extends knowledge on the context in which to study the impact of candidate genes on adiposity and disease risk. Grant code: FS/17/60/33474

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P24.006.A Deciphering how early life adiposity influences breast cancer risk using Mendelian randomization

Marina Vabistsevits, *George Davey Smith, Eleanor Sanderson, Tom Richardson, Bethan Lloyd-Lewis, Rebecca Richmond*

MRC Integrative Epidemiology Unit, University of Bristol, Bristol, United Kingdom.

Studies suggest that adiposity in childhood may reduce the risk of breast cancer in later life. The biological mechanism underlying this effect is unclear but is likely to be independent of adult BMI. In this work, we investigated 18 potential mediators of the protective effect in a Mendelian Randomization (MR) framework, reviewing hormones, reproductive, physical, and glycaemic traits.

Using data from publicly available GWAS, we designed an MR workflow to assess the causal role of potential mediators. We first evaluated whether the trait is affected by childhood BMI, and then whether it has a causal effect on breast cancer risk (two-step MR). We also assessed the independent effect of childhood BMI on breast cancer with each mediator taken into account (multivariable MR). Finally, we used mediation analysis to characterise the indirect effect of BMI via the mediators.

The results showed that although there is evidence of childhood BMI affecting most of the reviewed mediators, only IGF-1, testosterone, age at menarche and menopause, and mammographic density have an effect on breast cancer risk. However, multivariable MR showed that the protective effect of childhood BMI was not affected when accounted for those traits, suggesting a lack of evidence for mediation.

Our work presents a framework for systematic exploration of mediators in MR. We explored many plausible links between childhood adiposity and breast cancer risk, but none of the reviewed traits in this work accounted for the protective effect observed.

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P24.008.C Coeliac disease phenotypic variation unraveled by disease-susceptibility loci in a deeply phenotyped Finnish cohort

Juliana Xavier de Miranda Cerqueira[1], *Päivi Saavalainen*[2], *Kalle Kurppa*[3], *Pilvi Laurikka*[1], *Heini Huhtala*[4], *Matti Nykter*[5], *Lotta L. E. Koskinen*[2], *Dawit A. Yohannes*[2], *Elina Kilpeläinen*[6], *Anastasia Shcherban*[6], *Aarno Palotie*[6], *Katri Kaukinen*[1], *Katri Lindfors*[1]

[1]*Coeliac Disease Research Center, Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland,* [2]*Research Programs Unit, Immunobiology, and the Haartman Institute, Department of Molecular Genetics, University of Helsinki, Helsinki, Finland,* [3]*Center for Child Health Research, Tampere University and Tampere University Hospital, Finland, Tampere, Finland,* [4]*Faculty of Social Sciences, Tampere University, Tampere, Finland,* [5]*Laboratory of Computational Biology, Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland,* [6]*Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Helsinki, Finland.*

Introduction: Genome-wide association studies (GWAS) identified numerous genomic regions associated with coeliac disease (CeD). Their contributions to the heterogenous disease that varies considerably for unclear reasons remains largely unknown. We investigated whether CeD susceptibility variants (SNPs) are individually or cumulatively associated with distinct disease phenotypes. We also tested whether a polygenic risk score (PRS) could explain the phenotypic variation.

Material and methods: The phenotypic association of 39 non-HLA CeD SNPs was tested in 625 thoroughly phenotyped CeD patients and 1817 controls. To assess their cumulative effects a weighted genetic risk score (wGRS39) was built, and stratified by tertiles. In our PRS in cases, we took the summary statistics from a GWAS in CeD (24,269 participants) and tested their association with phenotypes at eight P value thresholds (P_T).

Results: Ten SNPs were associated with distinct phenotypes after correction for multiple testing ($P_{EMP2} \leq 0.05$). The TLR7/TLR8 locus was associated with disease onset before and the SH2B3/ATXN2, ITGA4/UBE2E3 and IL2/IL21 loci after 7 years of age. The latter three loci were associated with severe small bowel mucosal damage and SH2B3/ATXN2 with type 1 diabetes. Patients at the highest wGRS39 tertiles had OR > 1.62 for having CeD-related symptoms during childhood, severe small bowel mucosal damage, malabsorption, anaemia. PRS was only associated with dermatitis herpetiformis ($P_T = 0.2$, $P_{EMP2}=0.02$).

Conclusions: Independent CeD-susceptibility loci were associated with distinct phenotypes, suggesting that genetic factors play a role in determining the disease presentation. The increased number of CeD SNPs might predispose to a more severe disease course. Published: https://doi.org/10.1038/s10038-020-00888-5

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P24.009.D Tissue-specific expression data increases the power of gene-based collapsing analysis

Samuel Lewis[1], *Katherine Smith*[1], *Keren Carss*[1], *Peter MacCallum*[1], *Quanli Wang*[2], *Slave Petrovski*[1]

[1]*Centre for Genomics Research, Discovery Sciences, BioPharmaceuticals R&D, AstraZeneca, Cambridge, United Kingdom,* [2]*Centre for Genomics Research, Discovery Sciences, BioPharmaceuticals R&D, AstraZeneca, Waltham, MA, USA.*

Collapsing analysis compares the number of rare variants in each gene between cases and controls, to detect genes in which rare variants are significantly enriched or depleted for a given phenotype. This approach has given important insights into the genetic basis of many complex diseases. However, tissue-specific expression means that not all variants are expressed in all tissues. We tested the hypothesis that removing variants that are unexpressed in the relevant tissue could increase the power of

collapsing analysis. We developed TISSUE (TIssue Specific Screen Using Expression) -informed collapsing analysis, an approach that uses tissue-specific gene expression data from the GTEx database to identify and filter out isoforms found at low levels or unexpressed in the disease-relevant tissue prior to collapsing analyses. We tested TISSUE-informed collapsing analysis compared to standard collapsing analysis across 1,170 cardiovascular traits using exome data for 268,450 individuals from the UK Biobank. We found that TISSUE-informed collapsing analysis increases the statistical power for many associations. For example, the p value for the association between *TTN* and cardiomyopathy dropped from 1.70e-31 in standard collapsing analysis to 1.11e-38 in TISSUE-informed collapsing analysis. Overall, of the 83 significant associations ($p < 5.00e-08$) for the 1,170 traits tested with standard collapsing analysis, 37 (44.6%) became more significant using TISSUE-informed collapsing analysis. We conclude that by leveraging tissue-specific expression data, we can increase the power of collapsing analysis to identify associations between genes and disease. Future work will expand this study to investigate more phenotypes in 450,000 UK Biobank individuals.

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P24.010.A Reimagining the metabolic syndrome: A composite complex trait

Mads Engel Hauberg

Department of Clinical Genetics, Odense University Hospital, Odense C, Denmark.

Introduction: Hypertension, insulin resistance, abdominal obesity, decreased HDL, and high triglycerides co-occur as the metabolic syndrome at substantial health cost. Despite decades of research, problems discerning cause and effect have impeded our understanding of its pathophysiology.

Materials and Methods: Using GWASs we explored genetic correlations of the five aforementioned traits and 1218 additional GWAS traits using LD-score based genetic correlations. This was done to examine if the current definition was meaningful and to identify traits that could be substituted or added to the syndrome. We further used MAGMA to identify genetic loci, pathways, mouse/human phenotypes, drugs, tissues, and cells that were broadly associated with the syndrome or were uniquely associated with the different components of the syndrome.

Results: The metabolic syndrome traits were more correlated than traits in general ($P = 3.9E-6$). Several, often unexpected traits, significantly correlated with all components of the syndrome ($n = 314$). These included osteoarthritis, smoking, drinking, attention-deficit hyperactivity disorder, and various metabolites. At the gene level, genes associated with lipid/glucose metabolism, and adipocyte function showed associations across all aspects of the metabolic syndrome. Genes uniquely associated with a single trait of the syndrome were often well known from physiology and/or monogenic disease. Further analyses dissected the five constituent traits into different disease components. E.g. abdominal obesity had, in addition to the lipid/glucose component, also brain/behavioral component.

Conclusions: Jointly, this data-driven approach suggested endophenotypes and implicated both lipid/glucose metabolism and behavior in the syndrome as well as highlighting a genetic overlap with related monogenic traits and drug targets.

M.E. Hauberg: None.

P24.011.B Identification of the association between amylase gene copy number variations and pancreatic diseases

***Assel Arginbekova**, John Armour*

University of Nottingham,Queen's Medical Centre, Nottingham, United Kingdom.

Background: CNV (copy number variation) regions include a number of genes that can be associated with multifactorial human diseases. The amylase gene and its CNV can play an important role in the development of pancreatic diseases. Our study aims to explore the association between the CNV of the human amylase genes and diseases of the pancreas.

Materials and methods: In our ongoing pilot study, DNA was prepared from blood samples collected from 79 patients of European origin with diagnoses of acute and chronic pancreatitis, or pancreatic cancer. As a control, copy number was determined (417 for AMY2 and 472 for AMY1) from DNA samples of healthy people. Copy numbers of amylase genes were determined by the paralogue ratio test.

Results: Initial analysis showed a significant association ($P = 2.3x10^{-4}$) of an increase in the AMY1 copy number with the disease risk for pancreatic disease. The values in cases showed a 4-fold difference between high (copy numbers>5) and low copy numbers. For AMY2A/2B, higher copy numbers also showed a positive association ($P = 4.6x10^{-3}$). The analysis determined that the developing pancreatic diseases are more common from the age of 40 ($P = 6x10^{-5}$). At the same time, the development of pancreatic diseases has no association with the genders ($P = 0.1283$).

Conclusion: It is suggested that genes like AMY1, 2A/2B may correlate with the occurrence of pancreatitis as a disease modifier, and our work will continue to establish whether these associations are observed in a larger data set.

A. Arginbekova: None. **J. Armour:** None.

P24.012.C New genes for coronary artery disease detected via gene-based association analysis

***Irina Zorkoltseva**[1], Nadezhda Belonogova[1], Alexandra Shadrina[1], Anatoly Kirichenko[1], Yakov Tsepilov[1,2], Tatyana Axenovich[1,2]*

[1]Institute of Cytology and Genetics, Novosibirsk, Russian Federation, [2]Novosibirsk State University, Novosibirsk, Russian Federation.

Introduction: Genome-wide association analyses (GWAS) have identified more than 300 single nucleotide polymorphisms at 163 genetic loci associated with coronary artery disease (CAD). However, little is known about the causal CAD genes and the mechanisms of their action. We aimed to conduct a more detailed analysis of genes whose polymorphism may influence the risk of CAD.

Materials and Methods: Using the UK Biobank-based GWAS summary statistics, we performed a gene-based association analysis using four sets of genetic variants within a gene differing in their protein coding properties, and combination of three **Methods:** SKAT-O, PCA, and ACAT-V implemented in sumFREGAT package. We used a 'polygene pruning' procedure to eliminate the influence of strong GWAS signals outside the gene.

Results: We found 123 genes significantly associated with CAD. Using the extended sample, we validated 65 of these genes. Five of them, *CDK19, NCALD, ARHGEF12, HECTD4* and *PTPN11* were

located in four new loci. We prioritized the genes in known CAD loci. Usually, the gene closest to the top GWAS signal is interpreted as the causal gene. We showed that only 50% of validated genes were the closest to the top signal at each locus. For 19 known CAD loci, we showed that the probably causal genes are more distant from the top GWAS signal.

Conclusions: We identified 65 genes that contribute to CAD with their within-gene variants and prioritized genes in known CAD loci. This work was supported by the Ministry of Education and Science of the RF (project 0259-2021-0009/-17-117092070032-4).

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P24.013.D Genome-wide association study of estradiol levels, and the causal effect of estradiol on bone mineral density

***Daniel Schmitz**, Weronica E. Ek, Elin Berggren, Julia Höglund, Torgny Karlsson, Åsa Johansson*

Uppsala University, Uppsala, Sweden.

Estrogen is the primary female sex hormone and plays an important role for skeletal health in both sexes. Several enzymes are involved in estradiol metabolism but few genome-wide association studies (GWAS) have been performed to characterize the genetic contribution to variation in estrogen levels.

We performed GWAS for estradiol in males (N = 147,690) and females (N = 163,985) from UK Biobank (UKB). Estradiol was analyzed as a binary phenotype; above/below detection limit (175 pmol/L). We further estimated the causal effect of estradiol on bone mineral density (BMD) using Mendelian randomization.

We identified 14 independent loci associated ($P < 5x10^{-8}$) with estradiol levels in males, of which one (*CYP3A7*) was genome-wide, and another seven were nominally ($P < 0.05$) significant in females. In addition, one female specific locus was identified. Most candidate genes have functions that are relevant to estrogen metabolism and have not been discussed in relation to estradiol levels in previous GWAS. For example, *SRD5A2*, which encodes a steroid 5-alpha reductase that is involved in processing androgens, and *UGT3A1* and *UGT2B7* which encode enzymes likely to be involved in estradiol elimination. The allele that tags the O blood group at the *ABO* locus, was associated with higher estradiol levels.

We further applied Mendelian Randomization to identify a causal effect of estradiol on bone mass density, both in males (beta = 0.099, $P = 1.58x10^{-11}$) and, for the first time, in females (beta = 0.15, $P = 7.48x10^{-6}$). Our findings further support the importance of the body's own estrogen to maintain skeletal health in males and in females.

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P24.015.B Genetic variability of 6p22.1 in sepsis susceptibility: a fine mapping association study of the HLA

*Tamara Hernandez-Beeftink[1,2], Itahisa Marcelino-Rodriguez[1,3], **Eva Suarez-Pajes**[1], Megan L. Paynton[4], Luis A. Rubio-Rodríguez[5], Beatriz Guillen-Guio[1], Jose M. Lorenzo-Salazar[5], Almudena Corrales[1,6], M. Isabel García-Laorden[2,6], Miryam Prieto González[7], Aurelio Rodríguez-Pérez[8,9], Demetrio Carriedo[10], Jesús Blanco[6,11], Alfonso Ambrós[12], Elena González Higueras[13], Elena Espinosa[14], Arturo Muriel[11], David Domínguez[14], Louise V. Wain[4,15], Abelardo García de Lorenzo[16], José M. Añón[16], Javier Belda[17], Jesús Villar[2,6], Carlos Flores[1,5,6]*

[1]Research Unit, Hospital Universitario N.S. de Candelaria, Universidad de La Laguna, Santa Cruz de Tenerife, Spain, [2]Research Unit, Hospital Universitario de Gran Canaria Dr. Negrín, Las Palmas de Gran Canaria, Spain, [3]Instituto de Tecnologías Biomédicas (ITB), Universidad de La Laguna, Santa Cruz de Tenerife, Spain, [4]Department of Health Sciences, University of Leicester, Leicester, United Kingdom, [5]Genomics Division, Instituto Tecnológico y de Energías Renovables (ITER), Santa Cruz de Tenerife, Spain, [6]CIBER de Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Spain, [7]Servicio de Medicina Intensiva, Complejo Asistencial Universitario de Palencia, Palencia, Spain, [8]Department of Anesthesiology, Hospital Universitario de Gran Canaria Dr. Negrín, Las Palmas de Gran Canaria, Spain, [9]Department of Medical and Surgical Sciences, University of Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain, [10]Intensive Care Unit, Complejo Hospitalario Universitario de León, León, Spain, [11]Intensive Care Unit, Hospital Universitario Rio Hortega, Valladolid, Spain, [12]Intensive Care Unit, Hospital General de Ciudad Real, Ciudad Real, Spain, [13]Intensive Care Unit, Hospital Virgen de la Luz, Cuenca, Spain, [14]Department of Anesthesiology, Hospital Universitario N.S. de Candelaria, Santa Cruz de Tenerife, Spain, [15]National Institute for Health Research, Leicester Respiratory Biomedical Research Centre, Glenfield Hospital, Leicester, United Kingdom, [16]Intensive Care Unit, Hospital Universitario La Paz, Madrid, Spain, [17]Department of Anesthesiology and Critical Care, Hospital Clinico Universitario of Valencia, Valencia, Spain.

Introduction: Sepsis is a severe inflammatory response to infections with a high death rate. We previously conducted the first GWAS of copy number variations in 839 sepsis cases from the Gen-Sep Network and 1,453 controls, highlighting 6p22.1 as one of the significant loci linked to sepsis susceptibility. Due its importance in inflammatory and immunological diseases, here we performed a fine mapping of the Human Leukocyte Antigen (HLA) region contained in that locus.

Methods: We used SHAPEIT v2.837 for phasing the haplotypes and Impute2 to impute the classic HLA alleles, amino acids, and single nucleotide polymorphisms (SNPs). Association analyses were performed by logistic regressions using EPACTs v3.2.6. A Bonferroni correction was applied to identify significant classic HLA alleles ($p < 2.58E-4$) and amino acids ($p < 4.91E-5$). For SNPs, a significance threshold was established at $p < 1.50E-5$ based on the number of independent variants.

Results and conclusions: We analyzed a total of 194 classic HLA alleles, 1,019 amino acids and 10,919 SNPs. None of the classic HLA alleles ($p_{lowest} = 0.01$), amino acids ($p_{lowest} = 0.01$), or SNPs ($p_{lowest} = 9.84E-4$) were significantly associated with sepsis. Given the complexity of this phenotype, these results suggest that the HLA genetic variation is not a major driver of sepsis susceptibility or has a modest effect size.

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P24.017.D Meta-analysis of genome-wide association studies for N-glycosylation in 10,000 individuals

Sodbo Sharapov[1], *Yakov Tsepilov*[1,2], *Elizaveta Elgaeva*[1], *Evgeny Tiys*[1], *Arina Nostaeva*[1,3], *Frano Vuckovic*[4], *Irena Trbojević-Akmačić*[4], *Michel Georges*[5], *Karsten Suhre*[6], *Nishi Chaturvedi*[7], *Harry Campbell*[8,9], *Malcolm Dunlop*[9], *Frances Williams*[10], *Matthias B. Schulze*[11,12,13], *Tim Spector*[10], *Gordan Lauc*[4], *Yurii S. Aulchenko*[1]

[1]*Institute of Cytology and Genetics SB RAS, Novosibirsk, Russian Federation,* [2]*Novosibirsk State University, Novosibirsk, Russian Federation,* [3]*PolyKnomics, 's-Hertogenbosch, Netherlands,* [4]*Genos Glycoscience Research Laboratory, Zagreb, Croatia,* [5]*Unit of Animal Genomics, WELBIO, GIGA-R and Faculty of Veterinary Medicine, University of Liège, Liège, Belgium,* [6]*Department of Physiology and Biophysics, Weill Cornell Medicine-Qatar, Doha, Qatar,* [7]*MRC Unit for Lifelong Health & Ageing University College London, London, United Kingdom,* [8]*Colon Cancer Genetics Group, Cancer Research UK Edinburgh Centre, Medical Research Council Institute of Genetics & Molecular Medicine, Western General Hospital, The University of Edinburgh, Edinburgh, United Kingdom,* [9]*Centre for Global Health Research, Usher Institute of Population Health Sciences and Informatics, The University of Edinburgh, Edinburgh, United Kingdom,* [10]*Department of Twin Research and Genetic Epidemiology, School of Life Course Sciences, King's College London, London, United Kingdom,* [11]*Department of Molecular Epidemiology, German Institute of Human Nutrition Potsdam- Rehbruecke, Nuthetal, Germany,* [12]*German Center for Diabetes Research (DZD), Neuherberg, Germany,* [13]*Institute of Nutrition Science, University of Potsdam, Potsdam, Germany.*

Glycosylation is a common modification of proteins that influences their physical properties and biological function. Although changes in protein glycosylation are observed in many diseases, the examples of the use of glycans as biomarkers and therapeutic targets are limited. This is not in small part because the understanding of human glycome regulation in vivo is incomplete and fragmented.To bridge this gap, we performed the largest genome-wide association study of the human blood plasma protein N-glycosylation measured by ultra performance liquid chromatography in 10,765 people. We studied the association between 8.8 million genetic polymorphisms on human autosomes and 117 relative abundances of N-glycan structures. We discovered and replicated 31 associated loci, 16 of which are novel. The SBayesR prediction models that included on average 1,090,196 genetic polymorphisms explained up to 21% of glycan variance (36% of SNP-based heritability). To prioritize potentially causal genes in the established loci, we performed an *in silico* functional study. Eight loci contained genes coding enzymes with a known role in N-glycan biosynthesis, while 23 loci, including 16 novels, may contain regulators of protein glycosylation, including transcription factors, transporters, blood pQTLs for glycosylated proteins. Using a network-based multi-omics approach, we explored a functional network formed by the glycome-associated loci. Our results set the scene, provides data and hypotheses for future studies that will establish genes and gene networks involved in regulation of global, cell-, tissue-, and protein-specific pathways of protein glycosylation.This work was supported by a grant from the Russian Science Foundation (RSF) No. 19-15-00115.

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P24.019.B Effect of host genetics on the gut microbiome in 7,738 participants of the Dutch Microbiome Project

Esteban A. Lopera-Maya[1], *Alexander Kurilshikov*[1], *Adriaan van der Graaf*[1], *Shixian Hu*[1], *Sergio Andreu-Sánchez*[1], *Lianmin Chen*[1], *Arnau Vich-Vila*[1], *Ranko Gacesa*[1], *Trishla Sinha*[1], *Valerie Collij*[1], *Marjolein A. Y. Klassen*[1], *Laura A. Bolte*[1], *Milla F. Brandao-Gois*[1], *Pieter B. T. Neerincx*[1], *Morris A. Swertz*[1], *Lifelines Cohort Study, Hermie J. M. Harmsen*[1], *Cisca Wijmenga*[1], *Jingyuan Fu*[1], *Rinse K. Weersma*[1], *Alexandra Zhernakova*[1], *Serena Sanna*[2]

[1]*University of Groningen and University Medical Center Groningen, Groningen, Netherlands,* [2]*Institute for Genetic and Biomedical Research (IRGB), National Research Council (CNR), Cagliari, Italy.*

Host genetics are known to influence the gut microbiome, yet their role remains poorly understood. To robustly characterize these effects, we performed a genome-wide association study of 207 taxa and 205 pathways representing microbial composition and function within the Dutch Microbiome Project, a population cohort of 7,738 individuals from the northern Netherlands. Two robust, study-wide significant ($p < 1.89 \times 10^{-10}$) signals near the *LCT* and *ABO* genes were found to associate with multiple microbial taxa and pathways and were replicated in two independent cohorts. The *LCT* locus associations seemed modulated by lactose intake, while those at *ABO* could be explained by participant blood type in interaction with the secretor status determined by their *FUT2* genotype. Twenty-two other loci showed suggestive evidence ($p < 5 \times 10^{-8}$) of association with microbial taxa and pathways. At a more lenient threshold, the number of loci we identified strongly correlated with trait heritability, suggesting that much larger sample sizes are needed to elucidate the remaining effects of host genetics on the gut microbiome.

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P24.020.C Interest of the profile and admixed sample for genetic analysis of human facial morphology

Betty Bonfante[1], *Pierre Faux*[1], *Nicolas Navarro*[2,3], *Javier Mendoza-Revilla*[4,5], *Morgane Dubied*[2], *Charlotte Montillot*[2], *Emma Wentworth*[6], *Lauriane Poloni*[2,3], *Philip Jones*[7], *Ziyi Xiong*[8], *Sagnik Palmal*[1], *Fan Liu*[8,9,10], *Seth M. Weinberg*[11], *John R. Shaffer*[11], *Evie Stergiakouli*[12], *Laurence J. Howe*[12], *Pirro G. Hysi*[13], *Timothy D. Spector*[13], *Rolando Gonzalez-José*[14], *Lavinia Schüler-Faccini*[15], *Maria-Cátira Bortolini*[15], *Victor Acuña-Alonzo*[16], *Samuel Canizales-Quinteros*[17], *Carla Gallo*[4], *Giovanni Poletti*[4], *Gabriel Bedoya*[18], *Francisco Rothhammer*[19], *Christel Thauvin-Robinet*[2,20], *Laurence Faivre*[2,20], *Caroline Costedoat*[1], *David Balding*[7,21], *Timothy Cox*[22], *Manfred Kayser*[8], *Laurence Duplomb*[2], *Binnaz Yalcin*[2], *Justin Cotney*[6], *Kaustubh Adhikari*[23,7], *Andrés Ruiz-Linares*[1,7,24]

[1]*Aix Marseille University, Marseille, France,* [2]*Université Bourgogne Franche-Comté, Dijon, France,* [3]*PSL University, Paris, France,* [4]*Universidad Peruana Cayetano Heredia, Lima, Peru,* [5]*Institut Pasteur,*

Paris, France, [6]University of Connecticut Health, Farmington, CT, USA, [7]University College London, London, United Kingdom, [8]Erasmus MC University Medical Center Rotterdam, Rotterdam, Netherlands, [9]Beijing Institute of Genomics, Beijing, China, [10]University of Chinese Academy of Sciences, Beijing, China, [11]University of Pittsburgh, Pittsburgh, PA, USA, [12]University of Bristol, Bristol, United Kingdom, [13]King's College London, London, United Kingdom, [14]CONICET, Puerto Madryn, Argentina, [15]Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, [16]National School of Anthropology and History, Mexico City, Mexico, [17]UNAM-Instituto Nacional de Medicina Genómica, Mexico City, Mexico, [18]Universidad de Antioquia, Medellín, Colombia, [19]Universidad de Tarapacá, Arica, Chile, [20]CHU Dijon, Dijon, France, [21]University of Melbourne, Melbourne, Australia, [22]University of Missouri, Kansas City, MO, USA, [23]The Open University, Milton Keynes, United Kingdom, [24]Fudan University, Shanghai, China.

Introduction: here is an interest in finding genetic basis explaining human facial variations because of its impact in different fields such as forensic or biomedical sciences. Until now 9 genome-wide association studies (GWASs), mainly using samples of European ancestry, have reported about 50 genetic loci affecting human facial traits and only a dozen of these findings were replicated independently. Human face profile shows important variations between humans but no GWAS have focused on that particular phenotype so far. We performed various GWASs to identify variants impacting human profile traits in an admixed population.

Materials and Methods: We used a sample of more of 6,000 volunteers from Latin America gathered by the CANDELA consortium. We extracted 59 measurements from landmarks manually placed on photographs (2D) of the right profile of the volunteers and performed genetic analyses with nearly 9M imputed or genotyped SNPs.

Results: We found significant association of 32 traits with at least 1 (and up to 6) of 32 different genomic regions. These regions were enriched in regulatory elements specifically active during craniofacial development. Within those regions, 9 were not identified as regions affecting the non-pathological variation of human face. We were able to replicate 4 of these new associations in an independent sample. Among them is the VPS13B gene region that we also found to impact mouse facial morphology.

Conclusions: By investigating face profile variations in an admixed population, we were able to identify new regions associated with human face traits.

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P24.021.D Fine mapping of GWAS loci associated with multiple sclerosis to dissect the pathogenetic role of drug target genes

Miriam zuccalà*[*1]*, Alessandro Pizzino*[*1]*, Nadia Barizzone[1], Ferdinando Clarelli[2], Chiara Basagni[1], Melissa Sorosina[2], Elisabetta Mascia[2], Domizia Vecchio[3], Mattia Pozzato[4], Cristoforo Comi[3], Roberto Cantello[3], Vittorio Martinelli[5], Giancarlo Comi[6], Maurizio Leone[1,7], Massimo filippi[5,8], Filippo Martinelli-Boneschi[9], Federica Esposito[2], Sandra D'Alfonso[1]*

[1]Department of Health Sciences, University of Eastern Piedmont, Novara, and IRCAD (Interdisciplinary Research Center of Autoimmune Diseases), Novara, Italy, [2]Laboratory of Human Genetics of Neurological Disorders, Institute of Experimental Neurology (INSPE), Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Milan., Milano, Italy, [3]MS Centre, SCDU Neurology, AOU Maggiore della Carità, Novara, Italy, Novara, Italy, [4]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurology Unit and Multiple Sclerosis Center, Milan, Italy, Milano, Italy, [5]Neurology Unit, IRCCS San Raffaele Scientific Institute, Milan, Milano, Italy, [6]Institute of Experimental Neurology, San Raffaele Scientific Institute, Milan, Milano, Italy, [7]Unit of Neurology, Fondazione IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, FG, Italy, Milano, Italy, [8]Neuroimaging Research Unit, Institute of Experimental Neurology, Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Milan, Milano, Italy, [9]Dino Ferrari Centre, Neuroscience Section, Department of Pathophysiology and Transplantation (DEPT), University of Milan, Milano, Italy.

Multiple sclerosis (MS) is a complex autoimmune disease of the central nervous system. Recently large GWAShave uncovered more than 200 loci that independently contribute to disease pathogenesis. However, the specific functional mechanism by which they influence MS pathogenesis is still largely unknown. This study aims to dissect the deeper biological understanding of pathogenetic mechanisms of MS through combinations of human genetics association data and network biology approaches. To identify primarily associated variants in MS related regions, we performed a preliminary analysis of co-occurrence of drug target genes in the 201 known MS associated regions querying three drug databases. We selected for follow-up 11 regions (23 genes), that showed the highest ranking in a meta-analysis performed in large Italian sample sets. The level of significance of the genetic association with the pathological phenotype was analyzed through PhenoScannerto investigate if any SNPs show eQTL associations of significant expression and consequently quantify the regulatory effect of the risk variants. We also evaluated GEO, looking for two array expression datasets of MS patients vs healthy controls and the results were compared with what found on PhenoScanner. In conclusion, 4 regions have been selected from the 11 preselected, including 6 drug target genes for an upcoming functional evaluation. These 6 genes will be tested to evaluate their translational role for a better understanding of the main pathogenetic mechanisms of MS, the identification of new therapies and the design of clinical trials for MS treatments.

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P24.023.B A Genome-Wide Association Study in patients with sepsis supports the association of SAMD9 variant with 28-day survival

Tamara Hernandez-Beeftink*[1,2]*, Beatriz Guillen-Guio[1], Jose M. Lorenzo-Salazar[3], Almudena Corrales[4,5], M Isabel García-Laorden[2,5], Miryam Prieto González[6], Aurelio Rodríguez-Pérez[7,8], Demetrio Carriedo[9], Jesús Blanco[5,10], Alfonso Ambrós[11], Elena González Higueras[12], Elena Espinosa[13], Arturo Muriel[10], David Domínguez[13], Abelardo García de Lorenzo[14], José M. Añón[14], Javier Belda[15], Jesús Villar[2,5], Carlos Flores[1,3,5]*

[1]Research Unit, Hospital Universitario N.S. de Candelaria, Universidad de La Laguna, Santa Cruz de Tenerife, Spain, [2]Research Unit, Hospital

Universitario de Gran Canaria Dr. Negrin, Las Palmas de Gran Canaria, Spain, [3]Genomics Division, Instituto Tecnológico de Energías Renovables, Granadilla, Santa Cruz de Tenerife, Spain, [4]Research Unit, Hospital Universitario N.S. de Candelaria, Santa Cruz de Tenerife, Spain, [5]CIBER de Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Spain, [6]Servicio de Medicina Intensiva, Complejo Asistencial Universitario de Palencia, Palencia, Spain, [7]Department of Anesthesiology, Hospital Universitario de Gran Canaria Dr. Negrin, Las Palmas de Gran Canaria, Spain, [8]Department of Medical and Surgical Sciences, University of Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain, [9]Intensive Care Unit, Complejo Hospitalario Universitario de León, León, Spain, [10]Intensive Care Unit, Hospital Universitario Rio Hortega, Valladolid, Spain, [11]Intensive Care Unit, Hospital General de Ciudad Real, Ciudad Real, Spain, [12]Intensive Care Unit, Hospital Virgen de la Luz, Cuenca, Spain, [13]Department of Anesthesiology, Hospital Universitario N.S. de Candelaria, Santa Cruz de Tenerife, Spain, [14]Intensive Care Unit, Hospital Universitario La Paz, Madrid, Spain, [15]Department of Anesthesiology and Critical Care, Hospital Clinico Universitario of Valencia, Valencia, Spain.

Introduction: Sepsis is a severe systemic inflammatory response to infections that has a 20-30% mortality rate. To date, most genetic studies have focused on particular biological candidates. We performed the first genome-wide association study (GWAS) of 28-day survival in sepsis.

Methods. A GWAS was performed in 475 Europeans from the GEN-SEP cohort and 7.5 million imputed variants. Association analyses were conducted using Cox regression models, adjusting by gender, age, and the main two principal components of genetic variation. A replication was performed in 212 independent patients from the same cohort. Statistical significance was established at $p < 5.0E-8$ in the meta-analysis. Whole-blood transcriptomics from septic patients were assessed by focusing on genes linked to significant variants.

Results and conclusions. The GWAS identified three independent common variants associated with reduced 28-day survival, including an exonic variant in *SAMD9* (HR = 4.75, 95%CI = 2.86-7.89, $p = 1.77E-9$), which encodes the sterile alpha motif domain-containing protein 9, related with the inflammatory response to tissue injury. An upregulation of *SAMD9* expression in non-surviving septic patients ($p = 0.003$) was observed. In conclusion, we completed the first GWAS of 28-day survival in sepsis and identified novel candidate genes associated with reduced survival.

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P24.024.C Improving the representation of traits in the GWAS Catalog

***Elliot Sollis**[1], Annalisa Buniello[1,2], Maria Cerezo[1], Laura W. Harris[1], Elizabeth Lewis[1], Ala Abid[1], Peggy Hall[3], James Hayhurst[1,2], Lucia A. Hindorff[3], Sajo John[1], Aoife McMahon[1], Abayomi Mosaku[1], Santhi Ramachandran[1], Patricia Whetzel[1], Fiona Cunningham[1], Paul Flicek[1], Helen Parkinson[1]*

[1]European Molecular Biology Laboratory, European Bioinformatics Institute, Cambridge, United Kingdom, [2]Open Targets, Cambridge, United Kingdom, [3]Division of Genomic Medicine, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA.

The NHGRI-EBI GWAS Catalog (www.ebi.ac.uk/gwas) is a comprehensive resource of manually curated GWAS data (>11,000 studies as of February 2021), alongside full summary statistics for a growing proportion (currently ~30%). GWAS have been performed for an increasingly wide array of human traits. The most highly represented traits in the Catalog include blood biomarker measurements, diabetes, body mass index, schizophrenia, depression, Alzheimer's disease, breast carcinoma and bipolar disorder.

Traits are annotated in the Catalog using terms from the Experimental Factor Ontology (EFO), to enable standardisation across studies and interoperability with other resources. The Catalog is currently refining how traits are annotated, to improve scientific accuracy and enable more precise searching. All studies have a main trait (the variable under investigation), but many also include a background trait shared by all study participants (e.g. "Allergic rhinitis **in asthma**"). Previously, such traits were annotated with multiple EFO terms in the same field (i.e. allergic rhinitis, asthma). Here, we present new functionality allowing users to easily distinguish main and background traits to more accurately reflect the study design.

Investigating the range of traits in the Catalog also highlights differences in data availability between research areas, including the rate of summary statistics sharing. For example, summary statistics were available for 58% of ovarian cancer studies published since 2017, compared to only 6% of leukaemia studies. Strikingly, the rate for COVID-19 studies has been extremely high (99%). We invite summary statistics submissions and feedback to address underrepresented trait areas.

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P24.025.D Identifying host genetic determinants of HIV-1 reservoir markers reveals PTDSS2 and IRF7 as potential modifying factors in HIV-1 patients

Zhenhua Zhang

University Medical Center Groningen, Groningen, Netherlands.

Combination antiretroviral treatment (cART) cannot eradicate HIV-1 from the body due to the persisting virus. These HIV-1 reservoirs mainly comprise long-lived resting memory CD4+ T cells and show a high variability in size and activity. Therefore, the identification of host factors contributing to the variation could introduce new HIV-1 treatment strategies. We conducted a genome-wide quantity trait locus analysis to probe genetic variants linked to levels of cell-associated (CA)-HIV-1 DNA, CA-HIV-1 RNA and RNA:DNA ratio in whole blood CD4+ T cells from a HIV-1 patient cohort (207 Caucasians) under long-term suppressive cART (median 6.6 years). CA-HIV-1 DNA and CA-HIV-1 RNA levels were measured with droplet digital PCR assays and genotype information (522,455 SNPs) was retrieved via Infinium Global Screening array platforms. By additive linear regression models corrected by age, gender, CD4-nadir and HIV-1 duration, we identified one significant genetic association with CA-HIV-1

DNA (p-value $< 5 \times 10^{-8}$) and subsequently the PTDSS2 as a candidate gene. Also, four were found for RNA:DNA ratio (p-value $< 5 \times 10^{-7}$), which highlighted RNH1, IRF7, DEAF1 and RP11-1149M10.2 as candidates. Next, we validated the IRF7 SNV is significantly correlated with higher expression of the IRF7 gene in peripheral blood mononuclear cells from HIV-1 patients, supporting its functional role in HIV-1 infection. The presented data suggests CA-HIV-1 DNA levels and RNA:DNA ratio could be influenced by PTDSS2 and IRF7. These observations provide novel knowledge on the molecular mechanisms involved in HIV-1 reservoir establishment and/or maintenance and could indicate targets for future therapeutic strategies for HIV-1 reservoir in patients.

Z. Zhang: None.

P24.026.A Genetics of hand grip strength: novel insights from GWAS and PRS studies in young cohorts

Filippo Abbondanza[1], *Carol Wang*[2], *Andrew Whitehouse*[3], *Silvia Paracchini*[1]

[1]*University of St Andrews, St Andrews, United Kingdom,* [2]*University of Newcastle, Newcastle, Australia,* [3]*University of Western Australia, Western Australia, Australia.*

Introduction: Hand grip strength (HGS) is a predictor of cardiovascular disease and overall poor health outcomes and it is broadly used as a proxy for muscular strength and frailty. Genetic studies in adult cohorts found multiple markers associated with HGS and showed genetic correlation with bone density traits. Despite thesep findings, the genetics of HGS remains largely elusive and has never been explored in a cohort of young individuals.

Materials and Methods: We performed GWAS meta-analyses on three grip strength tests, namely with the right and left hand and the maximal score, in the ALSPAC and Raine cohorts (N_{ALSPAC} ~ 5,400, $N_{Raine} = 1{,}162$, age range 11-13 years). We followed standard protocols and tools for the GWAS and downstream analyses (GWAS: PLINK and ProAbel; GSEA: magma; meta-analysed: METAL; functional mapping: FUMA; SNP-heritability: LDSC; Polygenic risk scores: PRSice2).

Results: We reported a novel genome-wide significant hit for HGS (rs2968991, p = 2.34E-08) with the right hand and we replicated a previous association with *HOXB3* (p = 6.22E-08). The gene-set analyses found associations with *HOXB7* and *HOXB3*. We reported similar SNP-heritability than previous studies and highlighted a significant positive correlation between HGS and bone density and fracture risk PRS.

Conclusion: In addition to a novel association, we replicated associations previously reported both at pathway and gene specific level, suggesting a robust genetic component of HGS at different ages. The PRS results supported the relationship between HGS and bone density and fracture risk. This work was funded by the Royal Society.

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P24.028.C Associations of genetic variants and inflammation markers in the Lithuanian population

Toma Adomaitytė[1], *Ingrida Domarkienė*[2], *Dovilė Karčiauskaitė*[3], ***Laima Ambrozaitytė***[2], *Ingrida Kavaliauskienė*[2], *Raimonda Mekienė*[2], *Alina Urnikytė*[2], *Zita Aurelė Kučinskienė*[3], *Asta Maeikienė*[3], *Neringa Burokienė*[4], *Andrejus Coj*[3], *Vaidutis Kučinskas*[2]

[1]*Faculty of Medicine, Vilnius University, Vilnius, Lithuania,* [2]*Department of Human and Medical Genetics, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, Vilnius, Lithuania,* [3]*Department of Physiology, Biochemistry, Microbiology and Laboratory Medicine, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, Vilnius, Lithuania,* [4]*Clinic of Internal Diseases, Family Medicine and Oncology, Institute of Clinical Medicine, Faculty of Medicine, Vilnius University, Vilnius, Lithuania.*

Inflammation plays a key role in the development of complex diseases, such as cardiovascular diseases (CVDs). High-sensitivity C-reactive protein (hs-CRP) has been associated with CVDs and contributes to atherosclerotic plaques rupture. Levels of matrix metalloproteinases (MMPs) and their matrix-degrading activity are raised in areas of atherosclerotic plaques. The analysis of genetic factors is essential to understanding the pathogenesis of inflammation.

Study group included 435 individuals of the Lithuanian origin. Genotyping was performed using Illumina Infinium® HD SNP arrays. hs-CRP, MMP-9, IL-1β levels were determined in blood serum samples. Genes were selected based on interactions with hs-CRP, MMP-9 and IL-1β markers (37 genes, 382 SNPs). Gene set association analysis was performed using Fisher's exact test with Plink v1.9. Case/control groups were formed accordingly: individuals with hs-CRP levels >2.5mg/l were referred as cases (N = 111), <0.6 mg/l - as controls (N = 89); individuals with MMP-9 levels >84.74 ng/ml were referred as cases (N = 42), <38.33 ng/ml - as controls (N = 42); individuals with autoimmune diseases were referred as cases (N = 45), the healthy ones - as controls (N = 71). Frequencies of associated alleles and genotypes were compared with 1000 Genomes project data. Chi-squared test was used.

hs-CRP level was associated with *APCS* rs2121477 ($p = 0.0001$, OR = 0.12 (95% CI: 0.035-0.42)). MMP-9 level was associated with *CD44* rs378517 ($p = 0.00002$, OR = 4.1 (95% CI: 2.128-7.755)). rs2121477, rs378517 allelic frequencies differed from AFR, South and East Asians, AMR, also rs2121477 - from FIN, rs378517 - TSI ($p < 0.05$).

Our study identified new associations between inflammation markers hs-CRP, MMP-9 and genetic variants in the Lithuanian population.

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P24.029.D Genetic studies indicate that fetal - and not maternal - insulin resistance is associated with birth weight

Hermina Jakupović[1], *Mario García Ureña*[1], *Thorkild I. A. Sørensen*[1,2], *Tuomas O. Kilpeläinen*[1]

[1]*Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Copenhagen, Denmark,* [2]*Department of Public Health, Section of Epidemiology, Faculty of Health and Social Sciences, University of Copenhagen, Copenhagen, Denmark.*

Fetal and maternal insulin resistance have been linked to differences in birth weight. However, it is difficult to separate between fetal and maternal effects. We investigated whether the associations of fetal and maternal insulin resistance with birth weight are independent using a genetic approach. First, we performed a genetic correlation analysis between insulin resistance, measured by fasting insulin (FI) concentration (n = 98,210) or HOMA-IR (n = 46,186), and fetal and maternal effects on birth

weight (n = 210,076 to 298,412) using genome-wide summary results. Second, we studied whether genetic risk scores for insulin resistance, comprising 13 loci associated with FI or 53 loci associated with a combination of high FI, high triglycerides and low HDL cholesterol (FI-TG-HDL), contribute to fetal or maternal genetic effects on birth weight. We found a negative genetic correlation between insulin resistance and fetal effect on birth weight after adjusting for maternal genotype (FI: ρG -0.30 [95% CI -0.51, -0.09]; HOMA-IR: ρG -0.29 [95% CI -0.46, -0.11]). There was no significant genetic correlation between insulin resistance and maternal effect on birth weight. The genetic risk scores for insulin resistance showed a negative fetal effect on birth weight after adjusting for maternal genotype (FI: beta -0.12 [95% CI -0.25, -0.02]; FI-TG-HDL: beta -0.14 [95% CI -0.20, -0.07]), whereas no significant maternal effect on birth weight was seen after adjusting for fetal genotype. Our results indicate that genetic predisposition to higher fetal and not maternal insulin resistance is associated with birth weight.

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P24.030.A Genome-wide age at onset analysis discovers association of the *ApoE* locus with earlier onset of ischemic stroke

Joanna von Berg[1], *Braxton Mitchell*[2], *Steven Kittner*[2], *Jeroen de Ridder*[1,3], *Sander W. van der Laan*[4], *the SiGN consortium*

[1]*Center for Molecular Medicine, University Medical Centre Utrecht, Utrecht, Netherlands,* [2]*University of Maryland School of Medicine, Baltimore, MD, USA,* [3]*Oncode Institute, Utrecht, Netherlands,* [4]*Central Diagnostics Laboratory, Division Laboratories, Pharmacy, and Biomedical Genetics, University Medical Centre Utrecht, Utrecht, Netherlands.*

Genome-wide association studies (GWAS) of ischemic stroke (IS) commonly aim to identify genetic variants associated with lifetime risk of IS in the general population by applying a case-control design. A fundamentally different approach would be to investigate the age-of-onset (AAO) of IS, i.e. getting stroke earlier (or later) than the average of the case population. Such an approach would distinguish individuals at early risk from those at later risk, identify variants associated with disease timing, and point towards mechanisms involved in disease onset. As the prevalence of IS differs between men and women across age, there might be sexual dimorphic mechanisms involved in IS onset. We performed genome-wide linear regression in SiGN (without inclusion restrictions on age) to analyse AAO of IS limited to cases of European ancestry. To find potential sex-specific or sex-differential associations, we first analyse all cases regardless of sex (*all*) and then analyse stratified on sex. A variant in *ApoE* is significantly associated with AAO of IS in *all* (rs429358:T>C, p-value = $1.3e^{-8}$, beta = -1.6 years), with the largest effect size in women (p-value = $2.5e^{-7}$, beta = -2.4 years). A variant in *TRIB3* showed the largest and significant effect in men (rs67896217:C>T, p-value = $4.1e^{-9}$, beta = -2.4 years). Preliminary follow-up studies replicated the *ApoE* variant in external datasets. Our results indicate that considering the age at onset of ischemic stroke identifies genetic variants involved in disease accelerating processes.

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P24.031.B Association study of KLF1 gene variations with HbF and HbA2 levels in β-thalassemia carriers of Portuguese origin

Licinio Manco[1], *Celeste Bento*[2], *Luis Relvas*[2], *Tabita Maia*[2], *M Leticia Ribeiro*[2]

[1]*University of Coimbra, Coimbra, Portugal,* [2]*Centro Hospitalar e Universitario de Coimbra CHUC, Coimbra, Portugal.*

Introduction: Kruppel-like factor 1 (KLF1) is an erythroid specific transcription factor, that inhibits γ-globin expression through BCL11A. Several reports showed that mutations on *KLF1* gene are associated with increased levels of HbF and HbA_2. This study aims to examine the association between three *KLF1* common variants (rs3817621; rs79334031; rs2072597) and HbF and HbA2 levels in β-thalassemia carriers.

Materials and Methods: Eighty-one Portuguese β-thalassemia carriers (40 males, 41 females), aged 2 to 70 years (mean 32.9 years), were studied. HbF levels range from 0.2 to 9.5% and HbA2 levels from 3.5 to 6.1%. HbA2 and HbF levels were determined by HPLC. SNPs were genotyped by PCR-RFLP.

Results: Minor allele frequencies for SNPs rs3817621 (C), rs79334031 (A) and rs2072597 (C) were 0.213, 0.038 and 0.263, respectively. Basic simple linear regression, in the additive model, showed no significant association between the three SNPs and HbF levels (p >0.05). However, the 0.046 frequency haplotype CGT (rs3817621/rs79334031/rs2072597), reaches a significant association with increased levels of HbF (beta = 1.99; p = 0.024). A nominal association was found between the rs79334031 minor A-allele and increased levels of HbA_2, unadjusted (beta = 0.59; p = 0.037) and adjusted for age and sex (beta = 0.615; p = 0.028). Haplotype analyses showed a significant association with HbA_2 for the two di-nucleotide haplotypes combining the minor alleles rs3817621/rs79334031 CA (beta = 0.591; p = 0.036) and rs79334031/rs2072597 AC (beta = 0.921; p = 0.020), and a near-significant association for haplotype CAC (beta = 0.614; p = 0.075).

Conclusion: Our results indicate that *KLF1* variations could make a significant contribution to increase HbF and HbA_2 levels in β-thalassemia carriers.

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P24.032.C Association between indicators of lipid metabolism and genetic profile in the Lithuanian population

Neda Janonytė[1], ***Ingrida Domarkienė***[2], *Dovilė Karčiauskaitė*[3], *Zita Aurelė Kučinskienė*[3], *Neringa Burokienė*[4], *Asta Maeikienė*[3], *Andrejus Coj*[3], *Laima Ambrozaitytė*[2], *Ingrida Kavaliauskienė*[2], *Raimonda Mekienė*[2], *Alina Urnikytė*[2], *Vaidutis Kučinskas*[2]

[1]*Faculty of Medicine, Vilnius University, Vilnius, Lithuania,* [2]*Department of Human and Medical Genetics, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, Vilnius, Lithuania,* [3]*Department of Physiology, Biochemistry, Microbiology and Laboratory Medicine, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, Vilnius, Lithuania,* [4]*Clinic of Internal Diseases, Family Medicine and Oncology, Institute of Clinical Medicine, Faculty of Medicine, Vilnius University, Vilnius, Lithuania.*

Introduction: The most common disorders of lipid metabolism are LDL-hypercholesterolemia, HDL-hypocholesterolemia, hypertriglyceridemia. Identification of variants that determine lipid

metabolism should improve risk prediction, generate targets for pharmacologic intervention and is beneficial for further research.

Materials and Methods: DNA from 445 participants of Lithuanian origin was extracted from venous blood and genome-wide genotyping was performed. Individuals were divided by quartiles of metabolite concentrations into case and control groups according to: total cholesterol (<5.31 - control, >6.81 - case, mmol/l), triglycerides (<0.89, >1.77), HDL-cholesterol (>1.65, <1.16), ApoA1 (>1.81, <1.44, g/l), ApoB (<0.89, >1.22), lipoprotein(a) (<0.03, >0.23) levels and cardiovascular disease, hypertonia, stroke, diabetes phenotypes. Association analysis was performed using SNPs (487) of the protein-protein interacting genes (48). Allele and genotype frequencies of previously reported as associated 10 SNPs were compared among Lithuanian population and other populations from the 1000 Genomes Project. A chi-square test was performed using *PLINK*.

Results: This study identified new associations between: cholesterol levels and rs940806 ($p = 0.00718$), rs4947995 ($p = 0.03076$), rs12536061 ($p = 0.03076$), rs1111650 ($p = 0.03076$), rs10774519 ($p = 0.04462$); hypertonia and rs878847 ($p = 0.0263$). Frequency differences of previously associated SNPs were defined: between Lithuanians and FIN - 2, IBS - 1, CEU - 0, GBR - 0, TSI - 4, AFR - 8, AMR - 5, EAS - 6, SAS - 6. Differences between men and women, in ethnolinguistic regions of Lithuania were not detected.

Conclusions: Six new associations were identified, interpopulation differences in allele frequencies were determined. Population genetic structure regarding disease associated variants may benefit personalized medicine, clinical risk management, further research.

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P24.033.D Polygenic risk score of longevity predicts longer survival across an age-continuum

Niccolo Tesi[1], *Sven J. van der Lee*[1], *Marc Hulsman*[1], *Iris Jansen*[1], *Najada Stringa*[1], *Natasja M. van Schoor*[1], *Philip Scheltens*[1], *Wiesje M. van der Flier*[1], *Martijn Huisman*[1], *Marcel J. T. Reinders*[2], *Henne Holstege*[1]

[1]*Amsterdam UMC, location VUmc, Amsterdam, Netherlands,* [2]*Delft University of Technology, Delft, Netherlands.*

Studying the genome of centenarians may give insights into the molecular mechanisms underlying extreme human longevity and the escape of age-related diseases. Here, we set out to construct polygenic-risk-scores (PRS) for longevity and to investigate the functions of longevity-associated variants. Using a cohort of centenarians with maintained cognitive health ($N = 343$), a population-matched cohort of older-adults from five cohorts ($N = 2905$), and summary statistics data from a GWAS on parental longevity, we constructed a PRS including 330 independent variants that significantly discriminated between centenarians and older-adults ($p = 4.5x10^{-5}$, *APOE* variants excluded). This PRS was also associated with longer survival in an independent sample of younger individuals, ($p = 0.02$), leading up to a 4-year difference in survival based on common genetic factors only. We show that this PRS was, in part, able to compensate for the deleterious effect of the *APOE-ε4* allele, and that the variants included in the PRS were previously associated with several age-related diseases. Using an integrative framework, we annotated the 330 variants included in this PRS to the genes they associate with, and performed gene-set enrichment analysis. We found a significant enrichment for genes associated with cellular differentiation, developmental processes, and cellular response to stress. Together, our results suggest that the genome of healthy centenarians is enriched with a constellation of variants each exerting small advantageous effects on aging-related biological mechanisms that maintain overall health and decrease the risk of age-related diseases.

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P24.036.C Host genome variants influence on bacterial salivary composition in African admixed population

Antonio Espuela-Ortiz[1], *Esther Herrera-Luis*[1], ***Fabian Lorenzo-Diaz***[1,2], *Celeste Eng*[3], *Scott Huntsman*[3], *Michael A. Lenoir*[4], *Jose R. Rodriguez-Santana*[5], *Esteban G. Burchard* *[3,6], *Maria Pino-Yanes* *[1,7,8]

[1]*Genomics and Health Group, Department of Biochemistry, Microbiology, Cell Biology and Genetics, Universidad de La Laguna, San Cristóbal de La Laguna, Tenerife, Spain,* [2]*Instituto Universitario de Enfermedades Tropicales y Salud Pública de Canarias (IUETSPC), Universidad de La Laguna, San Cristóbal de La Laguna, Tenerife, Spain,* [3]*Department of Medicine, University of California San Francisco, San Francisco, CA, USA,* [4]*Bay Area Pediatrics, Oakland, CA, USA,* [5]*Centro de Neumología Pediátrica, San Juan, PR, USA,* [6]*Department of Bioengineering and Therapeutic Sciences, University of California San Francisco, San Francisco, CA, USA,* [7]*Instituto de Tecnologías Biomédicas (ITB), Universidad de La Laguna, San Cristóbal de La Laguna, Tenerife, Spain,* [8]*CIBER de Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Spain.*

Introduction: The relationship between human genetic variation and microbiota composition of different body sites has been recently started to be assessed through microbiota genome-wide association studies (mGWAS). However, similar to other genomic approaches, the main efforts have been targeted to study European descent populations, and little is known about other populations. This work aimed to explore the interplay between host genetics and the oral microbiota in two understudied populations, African Americans and Latinos.

Methods: A total of 114 African Americans from SAGE and 144 Hispanics/Latinos from GALA II were included in the analyses. Salivary bacterial 16S ribosomal RNA sequencing profiling and genomic data imputed using 1000 Genomes Project as reference panel were used to perform a mGWAS. For each genus, cohort-specific results from linear regression models corrected for age, sex, genetic ancestry and asthma were meta-analysed. Furthermore, in-silico functional analysis of genome-wide significant variants was carried out using public online databases.

Results: The most abundant genera detected in saliva were *Prevotella*, *Streptococcus*, *Haemophilus*, *Veillonella*, *Neisseria*, *Rothia*, and *Fusobacterium*. Genome-wide significant associations for variants located at 11q14.1 were found for *Streptococcus* (p-value $< 4.94x10^{-8}$). The top hit is an intronic variant of genes *AAMDC* and *INTS4* found to be a blood expression quantitative trait locus for *INTS4* (p-value $= 1x10^{-8}$), *AAMDC* (p-value $= 8x10^{-24}$), and *AQP11* (p-value $= 5x10^{-14}$), the latter being potentially involved in saliva volume regulation.

Conclusions: Genetic variation at 11q14.1 was associated with *Streptococcus* genus abundance in saliva in African-admixed populations. Funding: Funded by SAF2017-83417R MINECO/AEI/FEDER, UE, and a MICIU/ULL fellowship to AE-O.

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P24.037.D Mitochondrial genetic determinants of nuclear gene expression variation among four European ancestries

Josefina Lascano Maillard[1], *Halit Ongen*[1,2], *Emmanouil T. Dermitzakis*[1,2]

[1]*University of Geneva, Geneva, Switzerland,* [2]*Swiss Institute of Bioinformatics, Geneva, Switzerland.*

Introduction: Mitochondria are involved in different key aspects of cell homeostasis. Somatic mutations in the mitochondrial genome are linked to varying patterns of nuclear DNA methylation and acetylation, contributing to differential gene expression and to the development of particular diseases. Although mitochondrial sequence alteration was investigated in the pathological context, little is known about the effect of its variation among the population on phenotypic traits.

Materials and Methods: We assess the extent of genetic contribution from the mitochondrial genome to nuclear gene expression using RNAseq data of 358 lymphocytic cell lines (LCLs) from the *Geuvadis* project and matched *1000 Genomes* (1KG) mitochondrial genotypes from four different European ancestries. We further explore probabilistic relationships between nuclear genes associated with particular groups of mitochondrial variants, using Bayesian Networks.

Results: We find a total of 66 mito-nuclear expression quantitative trait-loci (eQTLs) involving 21 different mitochondrial variants and 65 unique nuclear genes. Mitochondrial eQTL variants are grouped into blocks of correlated genotypes ($r^2 > 0.8$) and the corresponding eQTL genes are enriched for terms related to mitochondrial genetic diseases and processes. Genes associated with language disorders, cancer, encephalomyelopathies and cardiomyopathies are observed in eQTLs with different mitochondrial genotypic groups and define pathways within Bayesian Networks.

Conclusions: Our results highlight probabilistic relationships between genes associated with variants from the same genotypic block, pinpointing potential players of mitochondrial traits and pathways. Beyond the link between pathogenic mutations and diseases development, our findings point towards an expanded role of the mitochondrial genome in human phenotypic variation.

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P24.039.B Genome-wide association study of nociceptive musculoskeletal pain treatment response in UK Biobank

Song Li[1], *Rianne van Boekel*[2], *Marieke Coenen*[1]

[1]*Department of Human Genetics, Radboud Institute for Health Sciences, Radboud university medical center, Nijmegen, Netherlands,* [2]*Department of Anesthesiology, Pain and Palliative Medicine, Radboud Institute for Health Sciences, Radboud university medical center, Nijmegen, Netherlands.*

Introduction: Pain management for nociceptive musculoskeletal pain (NMP) follows analgesic ladder, starting from nonsteroidal anti-inflammatory drugs (NSAID), followed by weak or strong opioid until pain is under control. However, effective pain treatment is hampered by inter-individual differences and unsatisfied pain treatment response (PTR) rates ranging from 34 to 79%. We aimed to elucidate the genetic background of PTR.

Materials and Methods: A genome-wide association study (GWAS) was performed in ~23,000 participants with NMP from the UK Biobank and a subtype analysis including only NMP with inflammatory symptoms. In both analysis, NSAID vs. opioid users were compared as a reflection of response to NSAIDs, adjusting for relevant co-variates. Genetic variants with p-values less than 1e-06 (suggestive threshold) were carried forward for functional annotation e.g. regulation of nearby genes or spatially close genes. Single nucleotide polymorphisms (SNP) heritability calculation was performed.

Results: We identified one genome-wide significant hit in an intergenic region, rs549224715 ($P = 3.88e-08$), and six signals passing the suggestive threshold. Subtype analysis did not yield suggestive results. Functionally related genes that are nearby or under the regulation of identified SNPs include *THBS4*, involved in spinal sensitization and neuropathic pain states; *CMYA5*, *SGCB*, *TMEM130*, associated with muscular dystrophy which is characterized by muscle pain. The SNP heritability is 16.36% ($P = 0.16$).

Conclusions: Our GWAS identified genes functionally related to pain and treatment of pain. The results warrant future validation.

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P24.040.C Same role but different actors: genetic regulation of post-translational modification of two different proteins

Arianna Landini[1], *Irena Trbojevic-Akmacic*[2], *Pau Navarro*[3], *Frano Vuckovic*[2], *Caroline Hayward*[3], *Tea Petrovic*[2], *Marija Vilaj*[2], *Gordan Lauc*[2,4], *James F. Wilson*[1,3], *Lucija Klaric*[3]

[1]*Usher Institute (University of Edinburgh), Edinburgh, United Kingdom,* [2]*Genos Glycoscience Research Laboratory, Zagreb, Croatia,* [3]*MRC Human Genetics Unit, Institute for Genetics and Cancer (University of Edinburgh), Edinburgh, United Kingdom,* [4]*Faculty of Pharmacy and Biochemistry (University of Zagreb), Zagreb, Croatia.*

Post-translational modifications (PTMs) are essential mechanisms used by cells to diversify protein functions and dynamically coordinate their signaling networks. Nevertheless, genetic regulation of protein N-glycosylation, similarly to other PTMs, is not yet fully understood. To determine whether N-glycosylation PTM is regulated by the same genetic mechanisms in different proteins, we performed genome-wide association meta-analysis of glycosylation of two proteins - transferrin (35 N-glycan traits, $N = 1890$) and immunoglobulin G (IgG) (24 N-glycan traits, $N = 2020$). In the first ever GWAS of transferrin N-glycosylation, we identified 10 significantly associated loci ($P < 1.43 \times 10^{-9}$), three of which (*TF, FOXI1* and *MSR1*) were never previously associated with the glycome of any protein, while three others were also associated with IgG glycosylation by previous studies. Two of the latter encoded the glycosyltransferase enzymes FUT6 and FUT8. Using colocalisation methods, we showed that while these two enzymes alter both proteins, there is strong support for a different causal variant in each gene influencing the glycosylation of each protein. Moreover, we also suggest that core fucosylation of the two proteins is regulated by different transcription factors, IKZF1 for IgG and HNF1A for transferrin. We thus begin to unravel the complex genetic regulation of the PTM of two common plasma proteins. Distinct transcription factors appear to regulate the same enzyme in different tissues, while different underlying causal variants in the same gene regulate glycosylation in a substrate-specific manner.

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P24.041.D Modifier genes in NF1: results of the first Genome-Wide Association Study in 1,333 patients

Laurence PACOT[1,2], *Audrey Sabbagh*[3], *Béatrice Parfait*[1,2], *Anne Boland-Auge*[4], *Delphine Bacq-Daian*[4], *Ingrid Laurendeau*[2], *Salah Ferkal*[5,6], *Audrey Briand-Suleau*[1,2], *Laurence Allanore*[5], *Jean-François Deleuze*[4], *Michel Vidaud*[1,2], *Dominique Vidaud*[1,2], *members of the NF France Network, Pierre Wolkenstein*[5,6,7], *Eric Pasmant*[1,2]

[1]*Service de Génétique et Biologie Moléculaires, Hôpital Cochin, DMU BioPhyGen, AP-HP.Centre-Université de Paris, Paris, France,* [2]*Institut Cochin, Inserm U1016 - CNRS UMR8104 - Université de Paris, CARPEM, Paris, France,* [3]*UMR 261 MERIT, IRD, Université de Paris, Paris, France,* [4]*CEA, Centre National de Recherche en Génomique Humaine, Evry, France,* [5]*Département de Dermatologie, AP-HP and Université Paris 12, Hôpital Henri-Mondor, Créteil, France,* [6]*CIC1430, Hôpital Henri-Mondor, AP-HP, Créteil, France,* [7]*EA7379, Université Paris Est Créteil, Créteil, France.*

Background: Neurofibromatosis type 1 (NF1) in an autosomal dominant disorder caused by loss-of-function mutations in the tumor suppressor gene *NF1*. A typical sign of the disease is the development of benign tumors of the peripheral nervous system, called neurofibromas (NFs), which can transform into malignant peripheral nerve sheath tumors (MPNSTs). Few *NF1* pathogenic variants have been correlated to a specific presentation of the disease, but intrafamilial phenotypic correlations and animal models suggested that part of the variable expressivity of NF1 could be explained by modifier genes.

Methods: NF1 patients were recruited through the NF-France network between 2003 and 2013 and molecularly characterized. All patients were phenotypically characterized using a standardized questionnaire. *NF1*-mutated patients were enrolled and genotyped with the Illumina OmniExpressExome chip. Imputation and quality controls were applied in 1,333 patients and with more than 7 million common genomic variants. The cohort was divided into a discovery ($n = 918$) and a replication ($n = 415$) samples. Association study focused on three major clinical features: cutaneous (cNFs), subcutaneous (scNFs), and plexiform (pNFs) neurofibromas.

Results: Genome-wide significance threshold (5.10^{-8}) was reached in the discovery sample on chromosome 9 for the pNFs phenotype. Twelve, three and four regions suggestive of association ($p < 10^{-6}$) were identified respectively for pNFs, cNFs, and scNFs. Evidence of replication was observed respectively for four, two and six loci.

Conclusion: Our study confirms the role of previously described modifier genes and points out new candidates to explore.

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P24.042.A List of 190 genes affecting neuroticism prioritized by a new gene-based association analysis framework

Nadezhda M. Belonogova[1], *Irina V. Zorkoltseva*[1], *Yakov A. Tsepilov*[1,2], *Tatiana I. Axenovich*[1,2]

[1]*Institute of Cytology & Genetics SB RAS, Novosibirsk, Russian Federation,* [2]*Department of Natural Sciences, Novosibirsk State University, Novosibirsk, Russian Federation.*

Introduction: Recent genome-wide association studies have reported that neuroticism is influenced by about 600 genes. Little is known about the mechanisms of their action. We aimed to conduct a more detailed analysis of genes that can regulate the level of neuroticism.

Materials and Methods: Using UK Biobank-based GWAS summary statistics, we performed a gene-based association analysis using four sets of within-gene variants, each set possessing specific protein-coding properties. To guard against the influence of strong GWAS signals outside the gene, we used a specially designed procedure called "polygene pruning".

Results: We identified 190 genes associated with neuroticism due to the effect of within-gene variants rather than strong GWAS signals outside the gene. Thirty eight of these genes are new. Within all genes identified, we distinguished two slightly overlapping groups obtained from using protein-coding and non-coding variants. Twenty three genes were identified using protein-coding SNPs, 16 of them included potentially pathogenic variants. For 14 neuroticism genes identified using noncoding SNPs, we found evidence of pleiotropy with gene expression. Using a bioinformatics analysis, we showed that candidate genes confirmed in our study are more relevant and specific to neuroticism in their functions than non-confirmed genes.

Conclusions: We prioritized the neuroticism genes and showed that the genes that contribute to neuroticism with their within-gene variants are the most appropriate candidate genes. This work was supported by the Russian Foundation for Basic Research (20-04-00464) and the Ministry of Education and Science of the RF (project 0259-2021-0009/AAAA-A17-117092070032-4 and the 5-100 Excellence Program).

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P24.043.B genetic analysis on multiple sclerosis multiplex families

Chiara Basagni[1], ***Nadia Barizzone***[1], *Rachele Cagliani*[2], *Laura Mendozzi*[3], *Cristina Agliardi*[3], *Ferdinando Clarelli*[4], *Elisabetta Mascia*[4], *Melissa Sorosina*[4], *Miriam Zuccalà*[1], *Domizia Vecchio*[5], *Cristoforo Comi*[5], *Vittorio Martinelli*[6], *Giancarlo Comi*[7], *Massimo Filippi*[6,8,9,10], *Federica Esposito*[4], *Manuela Sironi*[2], *Diego Forni*[2], *Filippo Martinelli-Boneschi*[11,12], *Franca Rosa Guerini*[3], *Sandra D'alfonso*[1]

[1]*Department of Health Sciences, University of Eastern Piedmont, Novara, and IRCAD (Interdisciplinary Research Center of Autoimmune Diseases), Novara, Italy,* [2]*Bioinformatics, Scientific Institute IRCCS E.MEDEA, Bosisio Parini, Lecco, Italy,* [3]*IRCCS FONDAZIONE DON GNOCCHI, Milan, Italy,* [4]*Laboratory of Human Genetics of Neurological Disorders, Institute of Experimental Neurology (INSPE), Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Milan, Italy,* [5]*Department of Translational Medicine, University of Eastern Piedmont, Novara, and IRCAD (Interdisciplinary Research Center of Autoimmune Diseases), Novara, Italy,* [6]*Neurology Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy,* [7]*7Institute of Experimental Neurology, San Raffaele Scientific Institute, Milan, Italy,* [8]*Neuroimaging Research Unit, Institute of Experimental Neurology, Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Milan, Italy,* [9]*Neurophysiology Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy,* [10]*Vita-Salute San Raffaele University, Milan, Italy,* [11]*Dino Ferrari Centre, Neuroscience Section, Department of Pathophysiology and Transplantation (DEPT), University of Milan, Milan, Italy,* [12]*Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neurology Unit and Multiple Sclerosis Center, Milan, Italy.*

Multiple Sclerosis (MS) is a complex genetic disease. Over 200 common susceptibility variants have been identified. To assess the role of rare variants, we studied one of the largest MS multiplex families with 5 affected members. A weighted Genetic Risk Score analysis suggested that the increased genetic risk in this family is

partly due to common known MS associated variants, suggesting a possible role of low and rare frequency variants shared among the affected members. Non-parametric linkage analysis identified 5 peaks of linkage (chromosome 2, 5, 9, 10, 21). These data have been coupled with WES and WGS data which allowed us to identify 10 genes (*KIF1A*, *AGXT*, *PTCH1*, *RNF20*, *OR13C4*, *DNTT*, *SH3PXD2A*, *HEMGN*, *ABCA1*, *TPTE*) harbouring coding variants, and 11 genes (*HDAC3*, *SH3RF2*, *SLC36A2*, *LINC01933*, *WHRN*, *PAPPA*, *LCOR*, *R3HCC1L*, *BTRC*, *ADD3*, *CTBP2*) harbouring non-coding variants, shared by all affected individuals, filtered for MAF and *in-silico* predictors. To further assess the possible role of these genes in MS susceptibility, we applied the same pipeline to WES of additional 28 Italian MS multiplex families which allowed us to replicate the identification of rare coding variants in MS patients in 5 genes (*DNTT, ABCA1*, *KIF1A*, *SH3PXD2A*, *TPTE)*. In conclusion, our study identified several genes harbouring rare coding and non-coding variants shared by affected members of a large MS family. The extension of these analyses to other cohorts of familial and sporadic MS patients and controls is ongoing to further assess the role in MS susceptibility of the identified rare variants.

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P24.044.C Omingenic model of control of N-glycosylation of immunoglobulin G

*Arina Nostaeva[1], Sodbo Sharapov[2], Gordan Lauc[3], Michel Georges[4], **Yury Aulchenko**[2]*

[1]Novosibirsk State University, Novosibirsk, Russian Federation, [2]Institute of Cytology & Genetics SD RAS, Novosibirsk, Russian Federation, [3]Genos Glycoscience, Zagreb, Croatia, [4]GIGA Institute, University of Liege, Liege, Belgium.

Introduction: The omnigenic model[1] proposes that genes controlling a complex trait could be divided into a core pathway that directly affects the trait and a periphery regulating the core. The model postulates that genetic variation in the core pathway is expected to explain a small part of the trait's heritability, while large part of heritability passes through trans-regulatory networks[2]. Here, we test the omnigenic model using an exemplar of N-glycosylation of immunoglobulin G (NgIgG); a trait, for which the core biochemical pathways are well understood.

Materials and Methods: We used published data[3] to partition SNP heritability. To reconstruct a network of genes that regulate the core N-glycosylation pathway and to estimate network's contribution to the heritability we measured NgIgG, CD19+ transcriptomes, and genomes of 200 people.

Results: Similar to[2] we observed that the strongest genetic associations lie in the regions containing genes from the core pathway; that genetic variation near the core pathway genes explains minor proportion of heritability; that periphery is enriched for genes with tissue-specific expression. We then established the NgIgG regulatory network and attempted to estimate the lower boundary of the proportion of heritability that can be accounted for by the trans-regulation of the core pathway.

Conclusions: Our results confirm and elaborate the predictions of the omnigenic model. Funding: The work was funded by the Russian Science Foundation grant number 19-15-00115. References: 1. Boyle et al. Cell (2017) 2. Sinnot-Armstrong et al. bioRxiv (2021) 3. Klaric et al. Sci. Adv. (2020)

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P24.045.D Genetic association analyses identify links between pelvic prolapse (PP) and connective tissue biology, cardiovascular and reproductive health

Natàlia Pujol Gualdo[1], *Maarja Lepamets[1], Kristi Läll[1], Riikka Arffman[2], Terhi Piltonen[2], Reedik Mägi[1], Triin Laisk[1]*

[1]Estonian Genome Centre, Institute of Genomics, University of Tartu, Tartu, Estonia, [2]PEDEGO Research Unit, Department of Obstetrics and Gynecology, University of Oulu, Oulu, Finland.

Pelvic prolapse (PP) is characterized by a descent of the pelvic organs into the vaginal cavity. PP affects around 40% of women after menopause and is the main indication for major gynecological surgery. However, the etiology of PP remains poorly understood. In this study, we present the largest genome-wide association study (GWAS) of PP to date. In the discovery phase, we meta-analyzed Icelandic, UK Biobank and the FinnGen R3 datasets, comprising a total of 20118 cases and 427426 controls, and seek for replication in an independent dataset from Estonian Biobank (7968 cases and 118895 controls). Finally, we conducted a joint meta-analysis combining these datasets. We looked at enrichment of association signal on gene-set, tissue and cell type level and examined associations with other phenotypes both at the genetic and phenotypic level. We further constructed polygenic risk scores (PRS) to explore options for personalized risk assessment and prevention. We detected 20 genetic loci significantly associated with POP ($p < 5 \times 10^{-8}$), from which 13 loci were novel and located near genes involved in urogenital tract development (rs7126322, $p = 4.35x10^{-15}$, *WT1*) and regulation of the oxytocin receptor (rs2267372, $p = 4.49x10^{-13}$, *MAFF*). Tissue and cell enrichment analyses underlined the role of the urogenital system and muscle smooth cells ($p < 0.00001$, FDR < 0.05). Furthermore, musculoskeletal disorders and cardiovascular disease were genetically correlated with POP. Analyzing the best PRS as quintiles showed association with incident disease (Harrell c-statistic = 0.614, SD = 0.006). Our study provides genetic evidence to improve the current understanding of PP pathogenesis and assesses a genetic tool for personalized risk stratification.

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P24.046.A Polygenic Risk Score Estimation in North-Western Russian Population

Valeriia Rezapova[1,2], *Nikita Kolosov[1,2], Oxana Rotar[1], Olga Freylikhman[1], Alexander Loboda[1,2], Olesya Melnik[1], Alexey Sergushichev[2], Andreas Gnirke[3], Christine Stevens[3], Alexander Meissner[4], Anna Kostareva[1], Alexandra Konradi[1], Mark J. Daly[3,5,6], Mykyta Artomov[1,2,3,6]*

[1]Almazov National Medical Research Centre, Saint-Petersburg, Russian Federation, [2]ITMO University, Saint-Petersburg, Russian Federation, [3]Broad Institute, Cambridge, MA, USA, [4]Max Planck Institute for Molecular Genetics, Berlin, Germany, [5]Finnish Institute for Molecular Medicine, Helsinki, Finland, [6]Massachusetts General Hospital, Boston, MA, USA.

Over the last decade, genome-wide association studies (GWAS) have discovered a substantial number of associated variants for many complex traits. However, even within European-centered GWAS data, there are local subpopulations significantly under-

represented in these studies. For example, Russians, being one of the largest ethnic groups among the Europeans, remained significantly under-represented in GWAS for years. We used a pilot genotyping cohort of 239 individuals from Saint-Petersburg to investigate phenotypic variance explained by polygenic risk scores (PRS) for 11 phenotypes. We used UK biobank (UKBB) GWAS summary statistics for corresponding phenotypes and selected optimal p-value thresholds for maximizing R^2 for PRS. Several strategies for PRS calculation were tested, including effects of genotype imputation and usage of sex-specific GWAS summary statistics. In addition, we compared R^2 estimates for polygenic risk models in a scenario when UKBB GWAS summary statistic is applied to target data from UKBB itself or Biobank Japan (BBJ). The best utility of UKBB GWAS was observed for UKBB participants, with predictive value for Russian-descent individuals taking an intermediate place between UKBB and BBJ. This work was financially supported by the Ministry of Science and Higher Education of the Russian Federation (Agreement No. 075-15-2020-901) to Al.K. and Broad Institute SPARC award to M.J.D. and A.M.

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P24.047.B A data-driven review of the genetic factors of pregnancy complications

Yury Barbitoff[1], Alexander Tsarev[2], Elena Vashukova[1], Evgeniia Maksiutenko[3], Ludmila Kovalenko[4], Larisa Belotserkovtseva[4], ***Andrey Glotov[1]***

[1]Ott's Institute of Obstetrics, Gynaecology and reproductology, St.-Petersburg, Russian Federation, [2]Bioinformatics Institute, St.-Petersburg, Russian Federation, [3]St. Petersburg State University, St.-Petersburg, Russian Federation, [4]Surgut State University, Surgut, Russian Federation.

Introduction: Over the recent years, many advances have been made in the research of the genetic factors of pregnancy complications. In this work, we use publicly available data repositories, such as the National Human Genome Research Institute GWAS Catalog, HUGE Gene Navigator, and the UK Biobank genetic and phenotypic dataset to gain insights into molecular pathways and individual genes behind a set of pregnancy-related traits.

Materials and Methods: Using both HuGE and GWAS Catalog data, we confirm that immune system and T-cell related pathways are one of the most important drivers of pregnancy-related traits.

Results: Pathway analysis of the data reveals that cell adhesion and matrisome-related genes are also commonly involved in pregnancy pathologies. We also find a large role of metabolic factors that affect not only gestational diabetes, but also the other traits. These shared metabolic genes include *IGF2*, *PPARG*, and *NOS3*. We further discover that the published genetic associations are poorly replicated in the independent UK Biobank cohort. Nevertheless, we find novel genome-wide associations with pregnancy-related traits for the *FBLN7, STK32B*, and *ACTR3B* genes, and replicate the effects of the *KAZN* and *TLE1* genes, with the latter being the only gene identified across all data resources.

Conclusions: Overall, our analysis highlights central molecular pathways for pregnancy-related traits and suggests a need to use more accurate and sophisticated association analysis strategies to robustly identify genetic risk factors for pregnancy complications.
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P24.048.C *LOXL1* risk variant suggests protective effect for exfoliation syndrome and glaucoma in the cohort of Lithuanian Chernobyl catastrophe liquidators

Gabriele Zukauskaite*, Ingrida Domarkiene, Ausra Matuleviciene, Evelina Marija Vaiteniene, Justas Arasimavicius, Vaidutis Kucinskas, Laima Ambrozaityte*

Vilnius University, Department of Human and Medical Genetics, Vilnius, Lithuania.

Introduction: Ionizing radiation is one of the environmental factors that is known to affect genomes and, therefore, challenge organisms to adapt and acquire new, more favorable traits or evoke the protective effect of the existing variants. Until recently, there were few studies analysing genomes of individuals that experienced high levels of ionizing radiation at a DNA sequence level. This study focuses on the search for the protective genome variation in the cohort of Lithuanian Chernobyl catastrophe liquidators (LCCLs).

Materials and Methods: Genome-wide genotyping using *Illumina Infinium OmniExpress-24 v1.3 Kit* of 93 LCCLs was performed. A list of potentially protective SNPs was selected and used for further statistical analysis. Genotype frequency, linkage disequilibrium and epistasis analysis were performed comparing genotyping data of LCCLs with the general Lithuanian population. *R studio* and *PLINK* software were used. Prepared questionnaire allowed to evaluate clinical phenotype data of LCCLs.

Results: We identified a genome variant rs3825942 in *LOXL1* (NM_005576.4:c.458G>A; Fisher's exact test, $p = 0.0192$) gene, which reached statistical significance in LCCLs group. Linkage disequilibrium and epistasis analysis identified genes *LHFPL3*, *GALNT6*, *PIH1D1*, *ANKS1B*, *METRNL* that may have a role in the genetic architecture of exfoliation syndrome (XFS) and glaucoma.

Conclusions. Ambiguous *LOXL1* variant is mostly considered as having negative effect on the development of XFS and glaucoma. The influence of recent positive selection, allele-flipping phenomenon and the fact, that only individuals with homozygous reference allele have glaucoma among LCCLs suggests otherwise.

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P24.049.D On the use of variant pathogenicity scores to improve rare variant association tests

Ozvan Bocher[1]*, Thomas Ludwig[1,2], Elisabeth Tournier-Lasserve[3], Gaëlle Marenne[1], Emmanuelle Génin[1]*

[1]Univ Brest, Inserm, EFS, UMR 1078, GGB, Brest, France, [2]CHRU Brest, Brest, France, [3]Inserm UMR-S1161, Paris, France.

Introduction: The implication of rare genetic variants in disease can be studied using association tests that aggregate rare variants in testing units and filter them based on predicted impact and allele frequencies. Testing units are usually the genes that are considered as a whole without accounting for the functional domains of encoded proteins. As an alternative, sliding window approaches have been proposed that avoid the pre-selection of

testing units but are computationally intensive and with performances that depend on window sizes. The filtering of variants is also often focused on coding parts, leaving out functionally relevant intronic variants.

Methods: We used pathogenicity scores observed in GnomAD to define testing units and to optimize the filtering of variants included in rare variant association tests. Using case-control exome sequence data on Moyamoya disease, we compared our proposed strategy to the classical gene-based analysis and to WGScan, a sliding window procedure. We evaluated the performances of these different strategies to detect the known signal on RNF213 by burden tests.

Results and conclusions: Our strategy and the sliding window approach were more efficient than the gene-based approach to detect the signal. They were able to delimit a restricted candidate region within the gene. Moreover, our region-based strategy to filter variants outperformed classical filtering strategies. These encouraging results suggest that a similar approach could also be used in the non-coding regions of the genome where we dramatically lack of functional annotations to define testing units and select qualifying variants.

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P24.051.B Genome-wide association study of smoking behaviors in a Chinese population of Taiwan

Ming-Wei Lin[1], *Chia-Yen Wu*[1], *Pei-Yu Weng*[1], *Ting-Yi Wu*[2], *Yi-Ying Wu*[1], *Chih-Kuan Lai*[3], *Kuang-Chieh Hsueh*[4], *I-Fan Liu*[5], *Hsin-Bang Leu*[6], *Li-Shiun Chen*[7], *Ivy Yi-Wen Tsai*[8], *Kung-Yee Liang*[9]

[1]*Institute of Public Health, National Yang Ming Chiao Tung University, Taipei, Taiwan,* [2]*Department of Health Care Management, Chang Gung University, Taoyuan, Taiwan,* [3]*Department of Family Medicine, Taipei Veterans General Hospital, Taipei, Taiwan,* [4]*Department of Family Medicine, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan,* [5]*Department of Cardiology, Cheng Hsin Hospital, Taipei, Taiwan,* [6]*Healthcare Center, Taipei Veterans General Hospital, Taipei, Taiwan,* [7]*Department of Psychiatry, Washington University, St. Louis, MO, USA,* [8]*Institute of Health Policy and Welfare, National Yang Ming Chiao Tung University, Taipei, Taiwan,* [9]*Institute of Population Health Sciences, National Health Research Institutes, Miaoli, Taiwan.*

Introduction: Tobacco smoking is one of the major risk factors for many chronic diseases and is the leading cause of preventable death in the world. Smoking behavior is a complex, multifactorial trait with both genetic and environmental factors contributing to the various phenotypes. The aims of this study were to conduct a genome-wide association study (GWAS) on smoking behaviors and to investigate the association between genes, smoking behaviors and their impact on the cardiovascular outcomes in a Chinese population of Taiwan.

Methods: We have enrolled 860 ever-smoking subjects recruited from the Healthcare Center and the Department of Family Medicine in the Taipei Veterans General Hospital, and the Department of Cardiology in the Cheng Hsin Hospital. Each participant was followed-up every six month by telephone interview with a structural questionnaire to obtain the information of their smoking status, smoking quantities, quitting attempt, and major cardiovascular events in subsequent one year. The Infinium CoreExome-24 BeadChips (Illumina, San Diego, CA) were used for the genome-wide association study. The PLINK program was used for the analysis of genome-wide association study.

Results: We identified several novel genes, including *RIT2, CLYBL, NFAM1, LRRC8E, FAM129B, HACD1, STK32A, CCDC88C, LINC01804, PCAT2, ASIC2, CNTNAP2*, were associated with smoking cessation at 6 months (with p-value 1×10^{-4}). Further studies to confirm our preliminary findings are warranted.

Conclusion: Our results identified several novel genes might be associated with smoking cessation in a Chinese population of Taiwan. Further study with larger sample is required to replicate our preliminary findings. Grant No: MOST 109-2314-B-010-045-

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P24.052.C Trans-ancestry GWAS of 118,780 individuals reveals biological mechanisms underlying the spatial QRS-T angle, a marker of arrhythmogenesis

William Jon Young[1], *Patricia B. Munroe*[1], *Larisa Tereshchenko*[2], *on behalf of the CHARGE consortium EKG working group*

[1]*Queen Mary University of London, London, United Kingdom,* [2]*Oregon Health and Science University, Portland, OR, USA.*

Background: The spatial QRS-T angle (spQRSTa), the angle between QRS and T-wave spatial vectors, is an established predictor for risk of arrhythmia and sudden cardiac death (SCD). However, the biological mechanism remains unclear. We sought to identify novel candidate genes associated with the spQRSTa, to improve our understanding of the underlying biology.

Methods: We performed a trans-ancestry meta-analysis of genome-wide association studies (15) imputed with 1000G / HRC reference panels, comprising 118,780 individuals (81.3% European, 10.7% Hispanic and 7% African). Genetic correlation with other electrocardiogram traits was estimated using linkage disequilibrium score regression. Gene prioritization and gene-set enrichment was performed using DEPICT.

Results: We identified 61 independent loci (58 novel) in the trans-ancestry meta-analysis and an additional novel locus in African and Hispanic ancestry-specific analyses. Percent variance explained by lead variants was 3.4% (2.5% increase by novel loci). Heritability in Europeans (UK-Biobank) was 22.3%. Genetic correlation with PR, QRS, JT and QT was low ($r_g = -0.06, 0.12$). Top gene-ontology terms included cardiac/muscle cell differentiation and chamber morphogenesis. At 11 loci, candidate genes had established relationships with cardiomyopathies in humans, including *MYH7* and *TNNT2*. At other loci, genes have roles in cardiac cell proliferation (*CENPA, ERBB4*), embryonic development (*PITX2, WNT2*), arterial development (*ALDH1A2*) and angiogenesis (*ANGPT1*).

Conclusions: These analyses highlight the sarcomeric assembly, cardiac development and vasculogenesis as key contributors to the spQRSTa. The findings provide insight into possible mechanisms underlying the association with risk of arrhythmogenesis and SCD. W.J.Young is funded by the Medical Research Council (Grant code MR/R017468/1)

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P24.053.D A Genome-Wide Association Study of Copy Number Variants of sepsis susceptibility

Itahisa Marcelino-Rodriguez[1,2], *Tamara Hernandez-Beeftink*[1,3], ***Luis A. Rubio-Rodríguez***[4], *Eva Suarez-Pajes*[1], *Beatriz Guillen-Guio*[1], *Jose M. Lorenzo-Salazar*[4], *Rafaela González-Montelongo*[4], *Almudena Corrales*[1,5], *M Isabel García-Laorden*[3,5], *Miryam Prieto-González*[6], *Aurelio Rodríguez-Pérez*[7,8], *Demetrio Carriedo*[9], *Jesús Blanco*[5,10], *Alfonso Ambrós*[11], *Elena González-Higueras*[12], *Elena Espinosa*[13], *Arturo Muriel*[10], *David Domínguez*[13], *Abelardo García-de-Lorenzo*[14], *José M. Añón*[14], *Javier Belda*[15], *Jesús Villar*[3,5], *Carlos Flores*[1,4,5]

[1]Research Unit, Hospital Universitario N.S. de Candelaria, Universidad de La Laguna, Santa Cruz de Tenerife, Spain, [2]Instituto de Tecnologías Biomédicas (ITB), Universidad de La Laguna, Santa Cruz de Tenerife, Spain, [3]Research Unit, Hospital Universitario de Gran Canaria Dr. Negrín, Las Palmas de Gran Canaria, Spain, [4]Genomics Division, Instituto Tecnológico y de Energías Renovables (ITER), Santa Cruz de Tenerife, Spain, [5]CIBER de Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Spain, [6]Servicio de Medicina Intensiva, Complejo Asistencial Universitario de Palencia, Palencia, Spain, [7]Department of Anesthesiology, Hospital Universitario de Gran Canaria Dr. Negrín, Las Palmas de Gran Canaria, Spain, [8]Department of Medical and Surgical Sciences, Universidad de Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain, [9]Intensive Care Unit, Complejo Hospitalario Universitario de León, León, Spain, [10]Intensive Care Unit, Hospital Universitario Rio Hortega, Valladolid, Spain, [11]Intensive Care Unit, Hospital General de Ciudad Real, Ciudad Real, Spain, [12]Intensive Care Unit, Hospital Virgen de la Luz, Cuenca, Spain, [13]Department of Anesthesiology, Hospital Universitario N.S. de Candelaria, Santa Cruz de Tenerife, Spain, [14]Intensive Care Unit, Hospital Universitario La Paz, Madrid, Spain, [15]Department of Anesthesiology and Critical Care, Hospital Clinico Universitario of Valencia, Valencia, Spain.

Introduction: Sepsis is a severe inflammatory response to infections and a major cause of death and healthcare expenditure worldwide. To date, no genome-wide association study (GWAS) has been conducted for sepsis susceptibility. Here, we provide the results of the first GWAS of Copy Number Variants (CNVs) in sepsis patients.

Methods: We conducted a one-stage GWAS of CNVs in 839 sepsis cases from the Gen-Sep Network and 1453 controls genotyped with the Axiom Genome-Wide CEU 1 Array (Thermo Fisher Scientific). We used the software PennCNV for variant calling, and ParseCNV and PLINK v1.9 for association testing of common CNVs (>1% frequency), adjusting the models for gender, age, and the first two principal components of genetic variation. A Bonferroni adjustment was applied correcting for the number of tested CNVs to declare significance ($p < 3.6E\text{-}5$).

Results and conclusions: Four CNVs, including one deletion in 6p22.1 ($p = 2.94E\text{-}5$) and three duplications in 1q21.1 ($p = 1.43E\text{-}8$), 9p11.2-q21.11 ($p = 8.31E\text{-}8$), and 15q11.1-11.2 ($p = 1.46E\text{-}05$) regions, were significantly associated with sepsis susceptibility. The deletion is found in the Human Leukocyte Antigen (HLA) region, which plays a central role in many inflammatory and immunological diseases. Our findings revealed structural variants associated with sepsis susceptibility and provided the basis for further fine-mapping studies at these loci.

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P24.054.A Genetic dissection of Cloninger's Temperament and Character Inventory, TCI, in an Italian isolate

Maria Pina Concas[1], *Alessandra Minelli*[2,3], *Susanna Aere*[4], *Fabrizio Serra*[1], *Anna Morgan*[1], *Beatrice Spedicati*[4], *Ginevra Morgante*[4], *Massimiliano Cocca*[1], *Massimo Gennarelli*[2,3], *Paolo Gasparini*[1,4], *Giorgia Girotto*[1,4]

[1]*Institute for Maternal and Child Health – IRCCS Burlo Garofolo, Trieste, Italy,* [2]*Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy,* [3]*Genetics Unit, IRCCS Istituto Centro S. Giovanni di Dio Fatebenefratelli, Brescia, Italy,* [4]*Department of Medicine, Surgery and Health Sciences, University of Trieste, Trieste, Italy.*

Personality has a fundamental role in underlying a series of psychiatric symptoms. Thus, an accurate assessment of personality and temperament is essential to search for possible correlations of higher-order behaviours with the underlying biology (genes).

Five hundred eighty-seven adult individuals (331 females-256 males) from Friuli Venezia Giulia Genetic Park were included in the study. All subjects completed the TCI scales to assess the four temperament dimensions (harm avoidance (HA), novelty seeking (NS), reward dependence (RD) and persistence (P)), and the three character dimensions (self-directedness (SD), cooperativeness (C) and self-transcendence (ST)). GWAS was performed for each scale using an additive model. Age, sex, education level (for NS, SD and C) and anxiety and depression status (for HA) were added as covariates.GWAS on TCI scales led to the identification of several genes with a significant or suggestive p-value, expressed in the brain and/or already associated with psychiatric disorders. In particular, for NS scales, *MAGI2* (p-value $= 9.14\text{x}10^{-8}$), broadly expressed in the brain and already associated with schizophrenia and major depressive disorder, and *CNTN4* (p-value $=3.39\text{x}10^{-7}$), previously associated with neurobehavioral phenotypes. As regards to HA scales, *BTBD3* ($p = 2.152\text{x}10^{-8}$) already linked to obsessive-compulsive disorder and *SIAH1* implicated in Parkinson's disease (p-value $=8.52\text{x}10^{-6}$). Concerning RD scales, *PARK2*, associated with young-adult onset Parkinson (p-value $= 8.27\text{x}10^{-9}$).

Results: demonstrated a series of GWAS significant/suggestive associations between TCI scales and genetic background. Additional studies are needed to further confirm present results and better elucidate the role of the genes here identified.

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P24.055.B High-resolution genetic maps provide new insights into mitochondrial dysfunction in Type 2 diabetes

Hannah Maude[1], *Winston Lau*[2], *Nikolas Maniatis*[2], *Toby Andrew*[1]

[1]*Imperial College London, London, United Kingdom,* [2]*University College London, London, United Kingdom.*

Introduction: Mitochondrial dysfunction is well-known to co-occur with Type 2 diabetes (T2D); a reflection of this is the fact that multiple T2D drugs and treatments target the mitochondria. However, there is an ongoing question as to what extent genetic mechanisms contribute to this process, particularly since T2D onset can itself impact mitochondrial function. Characterising these mechanisms is complicated by risk variants occurring in (1) large blocks of linkage disequilibrium (LD) and (2) non-coding regulatory elements.

Materials and Methods: Here, we use expression quantitative trait loci (eQTL) to investigate >260 genetic risk loci significantly associated with T2D risk in 5,800 T2D cases, for evidence of regulating the expression levels of nuclear-encoded mitochondrial genes (NEMGs) in adipose tissue. T2D loci and eQTL were mapped

using positional cloning by LD; an association mapping method which utilises high-resolution genetic maps and multiple genetic variants to offer increased power over conventional single-SNP tests of association.

Results: The expression of 50 NEMGs were associated with T2D risk loci. Independent cohorts validated these 50 NEMGs as being differentially expressed in individuals with T2D or insulin resistance, and in normoglycemic offspring with affected T2D parents, compared to unaffected controls. These 50 NEMGs showed more extreme differences in expression compared to all other NEMGs, validating them as a key subset of NEMGs associated with T2D.

Conclusions: Mitochondrial dysfunction in T2D may be driven by the altered expression of NEMGs. Furthermore, positional cloning by LD can be used to identify disease-associated loci, significant target genes and important biological insights.

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P24.056.C Evaluation of causal relationships between varicose veins of lower extremities and knee osteoarthritis using large-scale genetic data

*Alexandra S. Shadrina[1], **Elizaveta E. Elgaeva**[1], Maxim B. Freidin[2], Yakov A. Tsepilov[1,3]*

[1]Institute of Cytology and Genetics SB RAS, Novosibirsk, Russian Federation, [2]Department of Twin Research and Genetic Epidemiology, School of Life Course Sciences, King's College London, London, United Kingdom, [3]Novosibirsk State University, Novosibirsk, Russian Federation.

Introduction: Osteoarthritis (OA) is a highly prevalent musculoskeletal disorder and a leading cause of disability among older adults. Varicose veins (VVs) are a common venous pathology affecting over one third of adults worldwide. There is increasing evidence of the link between VVs of lower extremities and knee OA, but it is unclear if there are causal relationships between the disorders.

Materials and Methods: We performed a two-sample Mendelian randomization (MR) analysis using two pairs of publicly available genome-wide association study (GWAS) summary statistics for Europeans from UK Biobank (Gene ATLAS, N = 452,264) and FinnGen (N = 176,899). Each trait pair included non-overlapping GWAS datasets on "VVs of lower extremities" (I83 ICD-10 code) and "Gonarthrosis" (M17 ICD-10 code) gained from different biobanks. The MR was conducted using inverse variance weighted meta-analysis and Causal Analysis Using Summary Effect estimates (CAUSE) approach. All the tests were run in two directions.

Results: No statistically significant results were obtained and no concordance between the MR effect estimates (magnitude and direction) was observed.

Conclusions: We provided no support for causal relationships between VVs of lower extremities and knee OA. Conversely, even if the causality exists, its magnitude is not clinically significant since it has not been detected using such large datasets.

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P25 COVID-19

P25.001.D Renin-angiotensin system polymorphisms as potential modifier in COVID-19 outcome

*Maria Sabater Molina[1,2], Elisa Nicolas Rocamora[1], Asunción Iborra Bendicho[3], Elisa García Vázquez[4], Fernando Dominguez Rodriguez[5], Roberto Barriales Villa[6], Esther Zorio[7], **Cristina Gil Ortuño**[1], Carmen Muñoz Esparza[1], Ana Isabel Rodríguez[1], Ruben Jara Rubio[8], Antonio Moreno[3], Pedro Jorge Marcos Rodriguez[9], Pablo García Pavía[10], Juan Ramon Gimeno Blanes[1,2]*

[1]Inherited Cardiac Disease Unit. Universitary Hospital Virgen de la Arrixaca., Murcia, Spain, [2]Instituto Murciano de Investigación Biosanitaria, Murcia, Spain, [3]Universitary Hospital Virgen de la Arrixaca. Service of Microbiology, Murcia, Spain, [4]Hospital Universitario Virgen Arrixaca, Department of Infectious Disease, Murcia, Spain, [5]Department of Cardiology, Hospital Universitario Puerta de Hierro, Madrid, Spain, [6]Cardiology Department, Complexo Hospitalario Universitario A Coruña, A Coruña, Spain, [7]Unidad de Cardiopatías Familiares y Muerte Súbita, Servicio de Cardiología, Hospital Universitario y Politécnico La Fe, Valencia, Spain, [8]Universitary Hospital Virgen de la Arrixaca. Department of Intensive Care, Murcia, Spain, [9]Complejo Hospitalario Universitario de a Coruña, A Coruña, Spain, [10]Cardiac Surgery Department, Hospital Universitario Puerta de Hierro, Madrid, Spain.

Introduction: Infection by the SARS-Cov-2 virus produces in humans a disease of highly variable and unpredictable severity. SARS-CoV-2 requires the presence of the ACE2 protein to enter in the cell and ACE2 is a regulator of the renin-angiotensin system. Accordingly, we studied the associations between 8 polymorphisms from AGTR1, ACE2 and ACE genes and the severity of the disease produced by the SARS-Cov-2 virus.

Materials and methods: 316 Covid-19 patients with positive PCR were classified based on the severity of symptoms and group in two: outpatients (n = 103) and hospitalized patients (n = 213, which included hospitalized in plant, ICU and exitus patients). The sex, age and comorbidities data were collected and the genotype distributions of 8 different SNPs in all patients were analyzed.

Results: Three SNPs were associated with the hospitalization risk. While rs2106809 was associated with an increased risk of being hospitalized (T/T vs. T/C-C/C, OR = 1.91; p = 0.031), it was found that rs2074192 and rs5186 showed a protector effect (G/C vs. GG-AA, OR = 0.41; p = 0.032 and C vs. A, OR = 0.66; p = 0.037, respectively).As expected, the comorbidities increased the hospitalization risk, nevertheless the interaction analysis showed that determinate genotypes has more susceptibility than others and even some of them had a protective effect.

Conclusion: There are ACE2 and AGTR1 polymorphisms that could influence severity of phenotype Covid-19 and, moreover, it depends on gender and the presence of comorbidities.

Table 1. Baseline characteristics of patients.

	Total	Outpatients	Hospitalized	P-value
Age (mean ± SD)	59.96	52.69 ± 17.25	63.32 ± 15.92	0.000
Gender				
Male	198 (62.9%)	59 (57.3%)	139 (65.6%)	0.153
Female	117 (37.1%)	44 (42.7%)	73 (34.4%)	
No comorbidities	110 (35.9)	46 (48.9%)	64 (30.2%)	0.002
Comorbidities	196 (64.1%)	48 (51.1%)	148 (69.8%)	
HBP	105 (34.3%)	19 (20.2%)	86 (40.6%)	0.006
Cancer	29 (9.5%)	7 (7.4%)	22 (10.4%)	0.528
Chronic lung disease	27 (8.8%)	5 (5.3%)	22 (10.4%)	0.150
Diabetes	44 (14.4%)	4 (4.3%)	40 (18.9%)	0.001
Obesity	40 (13.1%)	5 (5.3%)	35 (16.5%)	0.006

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P25.002.A Correlation testing of severe Covid-19 with genetic risk for male androgenetic alopecia

Sabrina K. Henne[1], *Lara M. Hochfeld*[1], *Carlo Maj*[2], *Markus M. Nöthen*[1], *Stefanie Heilmann-Heimbach*[1]

[1]1Institute of Human Genetics, University of Bonn, School of Medicine & University Hospital Bonn, Bonn, Germany, [2]Institute for Genomic Statistics and Bioinformatics, University of Bonn, Bonn, Germany.

Male androgenetic alopecia (AGA) has been implicated as a putative risk factor in severe Covid-19 based on high incidences of AGA in male hospitalized Covid-19 patients. Androgen signaling, which plays a central role in AGA etiology, has been associated with severe Covid-19 symptoms in men. Given the epidemiological association and androgen-dependency of both traits, we hypothesized that a link between AGA and severe Covid-19 susceptibility may exist at the genetic level. To test this hypothesis, we performed polygenic risk score (PRS) analyses using data from the UK Biobank. In a first step, we established PRS based on SNPs associated with AGA ($p < 5 \times 10^{-8}$ and $p < 5 \times 10^{-5}$) in a large UK Biobank-based GWAS. No correlation was observed in sex-separated and age-corrected logistic regression of hospitalized vs non-hospitalized Covid-19 on these scores ($p>0.05$). A limitation of genome-wide PRS is the condensation of biologically distinct mechanisms and differing effect directions. Thus, we aimed at further dissecting a potential AGA/Covid-19 genetic association by generating pathway-based PRS (pPRS). SNPs were assigned to genes via positional mapping (distance < 10kb) and genes were mapped to pathways using KEGG, WikiPathways and Panther libraries. Logistic regressions were performed on pPRS per p-value-cutoff and library. Significant correlation of AGA-pPRS with Covid-19-severity was found for four pathways: Vitamin metabolism ($p_{FDR} = 0.02$), natural killer cell-mediated cytotoxicity ($p_{FDR} = 0.02$) and WNT-signaling ($p_{FDR} = 0.02$) in men as well as aryl hydrocarbon receptor-signaling ($p_{FDR} = 0.02$) in women. These data suggest that a shared genetic basis for Covid-19 and AGA exists in specific pathways, posing interesting links between the pathophysiologies of both traits.

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P25.003.B Emergency ethical and legal framework for genomic research during COVID-19 outbreak. Experience of three institutions from the Spanish National Health Service

Carmen Ayuso[1,2], *Javier Ruiz Hornillos*[3], *Lucia Llanos*[4], *Sandra Zazo*[5], *Lidia Fernández-Caballero*[1,2], *Federico Rojo*[5], *Esperanza García-Molina*[6], *Teresa Escámez*[6], *Encarna Guillen-Navarro*[7,8]

[1]Department of Genetics and Genomics, IIS - University Hospital Fundación Jiménez Díaz, Universidad Autónoma de Madrid (IIS-FJD-UAM), Madrid, Spain, [2]Centre for Biomedical Network Research on Rare Diseases, Instituto de Salud Carlos III, Madrid, Spain, [3]Department of Clinical Bioethics, University Hospital Infanta Elena, (IIS-FJD), Madrid, Spain, [4]Clinical Research Unit. Research Ethics Committee. IIS - University Hospital Fundación Jiménez Díaz, Universidad Autónoma de Madrid (IIS-FJD-UAM), Madrid, Spain, [5]Department of Pathology. FJD Biobank, IIS - University Hospital Fundación Jiménez Díaz, Universidad Autónoma de Madrid (IIS-FJD-UAM), Madrid, Spain, [6]Biobank Platform IMIB-Arrixaca, Murcia, Spain, [7]Medical Genetics Department. University Hospital Virgen de la Arrixaca, IMIB-Arrixaca, Universidad de Murcia, Murcia, Spain, [8]Spanish Bioethics Committee, Spanish Ministry of Health, ISCIII, Madrid, Spain.

Introduction: In March 2020, COVID-19 pandemic (WHO) and state of alarm in Spain (Spanish government) were declared. The emergency aroused an enormous research effort searching for diagnostic biomarkers, therapeutics and preventive strategies trying to solve this serious public health situation. However, the application of ethical principles and the request of the Informed Consent was not possible in the way in which it is regularly carried out.

Materials and Methods: We participated in a genomic association study aimed to stablish genetic host markers linked to COVID-19 prognosis. According to the project design, DNA and clinical data form 3,270 participants were collected. The protocol and the request for waiver of written informed consent, or waiver of IC at all, when not possible, were submitted to the local research ethic committees and informed to the institutional biobanks.

Results: In April 2020, the Spanish Bioethics Committee and Spanish Biobank Network released the recommendations on the ethical framework for research during and on COVID-19. Accordingly, the institutional committees approved the procedure, as well as, the waiver for written IC, in those cases where the infectious scenario made it not possible. Therefore, digital signature and a system to annotate the verbal consent were established in electronic clinical records.

Conclusions: COVID-19 pandemic fosters a necessary discussion to found out how to preserve the different ethical values -public health and individual rights- in a difficult scenario.

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P25.004.C Role of the FYVE and Coiled Coil Domain Autophagy Adaptor 1 in severity of COVID19 infection

Sandra P. Smieszek

Vanda Pharmaceuticals, Washington, DC, USA.

Coronaviruses remodel intracellular membranes to form specialized viral replication compartments, such as double-membrane vesicles where viral RNA genome replication takes place. Understanding the factors affecting host response is instrumental to design of therapeutics to prevent or ameliorate the course of infection. As part of explorative tests in hospitalized patients with confirmed COVID-19 infection participating in ODYSSEY trial, we obtained samples for WGS analysis as well as for viral genome sequencing. Based on our data, we confirm one of the strongest severity susceptibility locus thus far reported in association with severe COVID-19: 3p21.31 locus with lead variant rs73064425. We further examine the associated region. Interestingly based on LD analysis we report 3 coding mutations within one gene in the region of FYVE and Coiled-Coil Domain Autophagy Adaptor 1 (*FYCO1*). We specifically focus on the role of *FYCO1* modifiers and gain-of-function variants. We report the associations between the region and clinical characteristics in this severe set of COVID-19 patients. We next analyzed expression profiles of *FYCO1* across all 466 compounds tested. We selected only those results that showed a significant reduction of expression of *FYCO1*. The most significant candidate was indomethacin - an anti-inflammatory that could potentially downregulate *FYCO1*. We hypothesize that via its direct effects on the efficiency of viral egress, it may serve as a potent therapeutic decreasing the replication and infectivity of the virus. Clinical studies will be needed to examine the therapeutic utility of indomethacin and other compounds down-regulating *FYCO1* in COVID-19 infection and other strains of betacoronaviruses.

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P25.005.D Post-Mendelian genetic model in COVID-19

Alessandra Renieri[1,2,3], *Nicola Picchiotti*[4,5], *Elisa Benetti*[2], *Chiara Fallerini*[1,2], *Sergio Daga*[1,2], *Margherita Baldassarri*[1,2], *Francesca Fava*[1,2,3], *Kristina Zguro*[2], *Floriana Valentino*[1,2], *Gabriella Doddato*[1,2], *Annarita Giliberti*[1,2], *Rossella Tita*[3], *Sara Amitrano*[3], *Mirella Bruttini*[1,2,3], *Laura Di Sarno*[1,2], *Nicola Iuso*[1,2], *Diana Alaverdian*[1,2], *Giada Beligni*[1,2], *Susanna Croci*[1,2], *Maria Palmieri*[1,2], *Ilaria Meloni*[1,2], *Anna Maria Pinto*[3], *Chiara Gabbi*[6], *Stefano Ceri*[7], *Antonio Esposito*[7], *Pietro Pinoli*[7], *Francis P. Crawley*[8], *Elisa Frullanti*[1,2], *Francesca Mari*[1,2,3], *GEN-COVID Multicenter Study, Marco Gori*[4,9], *Simone Furini*[2]

[1]*Medical genetics, University of Siena, Siena, Italy,* [2]*Med Biotech Hub and Competence Center, Department of Medical Biotechnologies, University of Siena, Siena, Italy,* [3]*Genetica Medica, Azienda Ospedaliero-Universitaria Senese, Siena, Italy,* [4]*University of Siena, DIISM-SAILAB, Siena, Italy,* [5]*Department of Mathematics, University of Pavia, Pavia, Italy,* [6]*Independent Medical Scientist, Milan, Italy,* [7]*Politecnico di Milano, DEIB, Milano, Italy,* [8]*Good Clinical Practice Alliance-Europe (GCPA) and Strategic Initiative for Developing Capacity in Ethical Review-Europe (SIDCER), Leuven, Belgium,* [9]*Université Côte d'Azur, Inria, CNRS, I3S, Maasai, France.*

Host genetics is an emerging theme in COVID-19 and few common polymorphisms and some rare variants have been identified, either by GWAS or candidate gene approach, although an organic model is still missing. Here, we propose a new model, called "Post-Mendelian", that takes into account both common and rare germline variants applied in a cohort of 1,768 Italian SARS-CoV-2 positive individuals. Ordered logistic regression of clinical WHO grading on age, stratified by gender, was used to obtain a binary phenotypic classification. Genetic variability from WES was synthesized in several boolean representations differentiated according to allele frequencies and genotype effect. LASSO logistic regression was used for extracting relevant genes. We defined a group of common polymorphisms and a group of rare variants, corresponding to classical "threshold model". Extracted genes were gender specific. The combined results can be described as an integrative polygenic score (IPGS) computed as: (nmildness-nseverity) +F (mmildness-mseverity) where n is the number of common driver polymorphisms, m is the number of rare driver variants and F is a factor for appropriately weighing the more powerful rare variants. Low IPGS is indicative of severity (the smaller the number, the greater the severity). Further validations are needed in order to consolidate and refine the model which now has a prediction capacity of about 65%-70% and could be useful for personalised adjuvant therapy. MIUR "Dipartimenti di Eccellenza 2018-2020". Intesa San Paolo 2020 charity fund N. B/2020/0119. Tuscany Region "Bando Ricerca COVID-19 Toscana" 2020.

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P25.006.A Functional prediction and comparative population analysis of variants in genes for proteases and innate immunity related to SARS-CoV-2 infection

Kristel Klaassen, Biljana Stankovic, Branka Zukic, Nikola Kotur, Vladimir Gasic, Sonja Pavlovic, ***Maja Stojiljkovic***

IMGGE, University of Belgrade, Belgrade, Serbia.

Aiming to understand a host genetic component of COVID-19, susceptibility and resistance to SARS-CoV-2 infection, we focused on variants in genes encoding proteases and genes involved in innate immunity. Analysis of sequence data of coding regions of *FURIN, PLG, PRSS1, TMPRSS11a, MBL2* and *OAS1* genes in 143 unrelated individuals from Serbian population identified 22 variants with potential functional effect. *In silico* analyses (PolyPhen-2, SIFT, MutPred2 and Swiss-Pdb Viewer) predicted that 10 variants could impact the structure and/or function of proteins. These protein-altering variants (p.Gly146Ser in *FURIN*; p.Arg261His and p.Ala494Val in *PLG*; p.Asn54Lys in *PRSS1*; p.Arg52Cys, p.Gly54Asp and p.Gly57Glu in *MBL2*; p.Arg47Gln, p.Ile99Val and p.Arg130His in *OAS1*) may have predictive value for inter-individual differences in the response to the SARS-CoV-2 infection. Next, we performed comparative population analysis for the same variants using extracted data from the 1000 genomes project. Population genetic variability was assessed using delta MAF and Fst statistics. Our study pointed to 7 variants in *PLG, TMPRSS11a, MBL2* and *OAS1* genes with noticeable divergence in allelic frequencies between populations worldwide. Three of them, all in *MBL2* gene, were predicted to be damaging, making them the most promising population-specific markers related to SARS-CoV-2 infection. In conclusion, we identified 4 variants in genes encoding proteases (*FURIN, PLG* and *PRSS1*) and 6 in genes involved in the innate immunity (*MBL2* and *OAS1*) that might be relevant for the host response to SARS-CoV-2 infection. Acknowledgment: This work was supported by Ministry of Education, Science and Technological Development Republic of Serbia, EB: 451-03-68/2020-14/ 200042.

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P25.007.B Genetic prediction of morbidity and letal outcome for patients with severe COVID-19 pneumonia

Liliya Fishchuk[1], *Zoia Rossokha*[1], *Valeriy Pokhylko*[2], *Yuliia Cherniavska*[2], *Svitlana Tsvirenko*[2], *Serhii Kovtun*[3], *Natalia Medvedieva*[1], *Victoriia Vershyhora*[1], *Halyna Soloviova*[2], *Natalia Gorovenko*[4]

[1]*State Institution Reference-centre for Molecular Diagnostic of Public Health Ministry of Ukraine, Kyiv, Ukraine,* [2]*Ukrainian Medical Stomatological Academy, Poltava, Ukraine,* [3]*Poltava Regional Clinical Infectious Diseases Hospital of Poltava Regional Council, Poltava, Ukraine,* [4]*Shupyk National Medical Academy of Postgraduate Education, Kyiv, Ukraine.*

Introduction: It is well known that the main cause of critical complications in COVID-19 is an immune imbalance and systemic inflammatory response. According to the latest data, variants of *IL-6* and *VDR* genes which encode the relevant components of the immune system can affect on the pathogenesis of this disease. The purpose of our study was to evaluate the impact of *IL-6* (G174C, rs1800795) and *VDR* (TaqI or T1056C, rs731236; BsmI or G283A, rs1544410) genes variants on the course of severe COVID-19 pneumonia.

Materials and Methods: The study group included 31 patients (15 women and 16 men) with diagnosis "viral COVID-19 pneumonia" treated at the intensive care unit. Out of 31 hospitalized patients, 6 patients died of complications caused by

COVID-19. Determination of the *IL-6* and *VDR* genes variants was used PCR-RFLP.

Results: It was identified following frequency of genotypes for G174C variant of *IL-6* gene: GG - 19.4%, GC - 41.9%, CC - 38.7%. Comparing the obtained frequencies with the population ones, it was found a significant increase in the frequency of CC genotype and C allele in study group (38.7%. vs 12.0% and 0.6 vs 0.4, respectively). It was found that in the deceased compared to the non-deceased patients was significantly increased the frequency of heterozygous genotypes TC and GA (100% vs 44%; 100% vs 28%,$p < 0.05$, respectively) for TaqI and BsmI variants of the *VDR* gene.

Conclusions: The investigated variants of the *IL-6* and *VDR* genes may be the genetic predictor of morbidity and lethal outcomes in patients with COVID-19.

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P25.008.C *HLA-A*11:01:01:01, HLA*C*12:02:02:01-HLA-B*52:01:02:02,* age and sex are associated with severity of Japanese COVID-19 with respiratory failure

Seik-Soon Khor[1], *Yosuke Omae*[1], *Nao Nishida*[2], *Masaya Sugiyama*[2], *Noriko Kinoshita*[3], *Tetsuya Suzuki*[3], *Michiyo Suzuki*[3], *Satoshi Suzuki*[4], *Shinyu Izumi*[5], *Masayuki Hojo*[5], *Norio Ohmagari*[3], *Masashi Mizokami*[2], *Katsushi Tokunaga*[1]

[1]*Genome Medical Science Project, National Center for Global Health and Medicine Hospital, Tokyo, Japan,* [2]*Genome Medical Science Project, National Center for Global Health and Medicine Hospital, Chiba, Japan,* [3]*Disease Control and Prevention Center, National Center for Global Health and Medicine Hospital, Tokyo, Japan,* [4]*Biobank, National Center for Global Health and Medicine, Tokyo, Japan,* [5]*Department of Respiratory Medicine, National Center for Global Health and Medicine Hospital, Tokyo, Japan.*

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus causing coronavirus disease 2019 (COVID-19) was announced as an outbreak by the World Health Organization (WHO) in January 2020 and as a pandemic in March 2020. The majority of infected individuals have experienced no or only mild symptoms, ranging from fully asymptomatic cases to mild pneumonic disease. However, a minority of infected individuals develop severe respiratory symptoms. The objective of this study was to identify susceptible HLA alleles and clinical markers for the early identification of severe COVID-19 among hospitalized COVID-19 patients. A total of 137 patients with mild COVID-19 (mCOVID-19) and 53 patients with severe COVID-19 (sCOVID-19) were recruited from the Center Hospital of the National Center for Global Health and Medicine (NCGM), Tokyo, Japan for the period of February-August 2020. High-resolution sequencing-based typing for eight HLA genes was performed using next-generation sequencing. In the HLA association studies, HLA-A*11:01:01:01 [$P_c = 0.013$, OR = 2.26 (1.27-3.91)] and HLA-C*12:02:02:01-HLA-B*52:01:01:02 [$P_c = 0.020$, OR = 2.25 (1.24-3.92)] were found to be significantly associated with the severity of COVID-19. After multivariate analysis controlling for other confounding factors and comorbidities, HLA-A*11:01:01:01 [P = 3.34E-03, OR = 3.41 (1.50-7.73)], age at diagnosis [P = 1.29E-02, OR = 1.04 (1.01-1.07)] and sex at birth [P = 8.88E-03, OR = 2.92 (1.31-6.54)] remained significant. Early identification of potential sCOVID-19 could help clinicians prioritize medical utility and significantly decrease mortality from COVID-19.

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P25.009.D Analysis of the distribution of the rs657152 and rs11385942 associated with the severe course of COVID-19 in the populations of Northern Eurasia

Natalia V. Ekomasova[1,2], *Murat Dzhaubarmezov*[1,2], *Anastasia Kazantseva*[1], *Liliya Gabidullina*[3], *Denis Antonov*[3], *Ekaterina Tokareva*[3], *Elza Khusnutdinova*[1,2]

[1]*Institute of Biochemistry and Genetics - Subdivision of the Ufa Federal Research Centre of Russian Academy of Sciences (IBG UFRC RAS), UFA, Russian Federation,* [2]*Bashkir State University, Ufa, Russian Federation,* [3]*Bashkir State University, UFA, Russian Federation.*

According to previous GWAS involving 1980 samples from the Western European populations of Spaniards and Italians, a severe course of COVID-19 (respiratory failure) was associated with rs11385942 and rs657152 (Ellinghaus et al., 2020). We analyzed 517 individuals from 10 populations of Northern Eurasia (Bashkirs from the Arkhangelsk region of the Republic of Bashkortostan, Bashkirs from the Burzyansky region of the Republic of Bashkortostan, Tatars, Chuvash, Balkars, Karachays, Kalmyks, Mordvins, Evens and Kazakhs). We found that the highest frequency of the GA-insert rs11385942 was characteristic for the populations of the Arkhangelsk Bashkirs and Karachays with frequencies of 11.7% and 11.2%, respectively. In the Even population this insert was absent. The rs657152 A-allele was observed with the highest frequency in the populations of Burzyan Bashkirs (53.12%) and Kazakhs (47.5%), while the lowest frequency was shown in Evens (33.3%). Ministry of Science and Higher Education of the Russian Federation (FZWU-2020-0027).

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P25.010.A Pharmacogenomics landscape of COVID-19 therapy response in Serbian population and comparison with worldwide populations

Branka Zukic, *Biljana Stankovic, Nikola Kotur, Vladimir Gasic, Kristel Klaassen, Bojan Ristivojevic, Maja Stojiljkovic, Sonja Pavlovic*

Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia.

Drug repurposing became important when treating COVID-19 patients. With limited time to test individual pharmacogenomics markers, population pharmacogenomics could help in predicting a higher risk of developing adverse reactions and treatment failure in COVID-19 patients. Aim of our study was to identify pharmacogenomics markers associated with drugs recommended for COVID-19 treatment, chloroquine/hydroxychloroquine, azithromycin, lopinavir and ritonavir, in Serbian population and other world populations. Genotype information of 143 individuals of Serbian origin was extracted from database previously obtained using TruSight One Gene Panel (Illumina). Genotype data of individuals from different world populations were extracted from the 1000 Genome Project. Fisher's exact test was used for comparison of allele frequencies. We have identified 11 potential pharmacogenomics markers in 7 pharmacogenes relevant for COVID-19 treatment. Based on high alterative allele frequencies in population and the functional effect of the variants, *ABCB1* rs1045642 and rs2032582 could be relevant for reduced clearance of azithromycin, lopinavir and ritonavir drugs and *UGT1A7* rs17868323 for hyperbilirubinemia in ritonavir treated COVID-19 patients in Serbian population. *SLCO1B1* rs4149056 is a potential marker of lopinavir response, especially in Italian population. Our results confirmed that pharmacogenomics profile of African population is different from the rest of the world. Considering population specific pharmacogenomics landscape, preemptive testing for pharmacogenes relevant for drugs used in COVID-19 treatment could contribute to better understanding of the inconsistency in therapy response and could be applied to improve the outcome of the COVID-19 patients. Ministry of Education, Science and Technological Development Republic of Serbia, (EB: 451-03-68/2020-14/200042) supported this work.

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P25.011.B EMCOVID19-ReCovER study: clinical and psychosocial impact of COVID-19 on the pediatric population diagnosed with rare diseases

Lourdes Vega Hanna, *Dídac Casas Alba, Leticia Pías, Mercè Bolasell, Francesc Palau, Antonio Federico Martínez Monseny*

Hospital Sant Joan de Déu d'Esplugues, Barcelona, Spain.

Introduction: The COVID-19 pandemic has favored the convergence of several stressors, including those associated with stay-at-home lockdowns. These stressors could aggravate the somatic, emotional and behavioral problems present in pediatric population with rare diseases (RD). We have developed a strategy to detect and prevent the issues derived from such stressors.

Methods: Pediatric patients diagnosed with RD associated with high physical and psychological comorbidities attended at a tertiary hospital were recruited (August to October 2020). Relatives of the participants were asked to complete a structured online questionnaire, including socio-demographic and clinical data. Emotional and behavioral outcomes have been assessed using PSC and ABC-C scores.

Results: Seventy-three patients with syndromic RD were included, being 22q11 deletion, Noonan and Prader-Willi syndromes the most frequent. During the widespread lockdowns, we did not observe a higher rate of emotional or behavioral problems compared to baseline. A modest improvement was observed when using the ABC-C score ($p = 0.00$). An increased risk of COVID-19 infections was not observed. An interruption of the multidisciplinary care for these patients has been apparent. The use of telemedicine to substitute their scheduled visits has been welcomed and perceived positively. The suspension of physical and speech therapies has elicited a negative perception on parents about the evolution of these patients.

Conclusions: Home confinement does not seem to have had a negative impact on pediatric population with RD. More studies are needed to identify the protective factors. Telemedicine has proven useful and opens up new strategies to multidisciplinary follow-up in RD.

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P25.012.C Incidence and severity of COVID-19 in rare disease populations

Zoe C. Wong, *Yi-Ping Fu, Blanche P. Alter, Lisa J. McReynolds, Manfred Boehm, Alexandra F. Freeman, Bernadette R. Gochuico, Alisa M. Goldstein, Mary L. McMaster, Neil E. Caporaso, Nehal N. Mehta, Kenneth Olivier, D. Rebecca Prevots, Armin Raznahan, Sharon A. Savage, Payal P. Khincha, Douglas R. Stewart, Swee Lay Thein, Brigitte C. Widemann, Andrea M. Gross, Neal S. Young, Karen Usdin, Audrey Thurm, Robert B. Hufnagel, Tiffany Powell-Wiley, Beth A. Kozel*

National Institutes of Health, Bethesda, MD, USA.

Introduction: By February 2021, 108 million people had been infected by SARS-CoV-2, a disease associated with a worldwide 2.2% mortality rate. Epidemiological studies show sociodemographic-mediated differences in outcomes, but less is known about case incidence and severity in rare disease populations.

Methods: Online survey information was collected from 1,614 individuals with rare, common, or no known health conditions over two months, ending November 2020. Participants reported rare diagnosis status in addition to COVID-19 symptoms, test results, and level of care required following COVID-19 diagnosis.

Results: Participants were female (62.4%) and mostly Caucasian (86.9%), aged 0.1-90 years ($M = 37.04$ y, $SD = 22.6$). 88% had a rare condition. Altogether, 51 respondents (3%) tested positive for COVID-19. Of these, six utilized hospital/emergency room services and one required intensive care. Rare conditions associated with more severe outcomes included Marfan syndrome, Fragile X, Dyskeratosis Congenita, Patterned Macular Dystrophy, and Vascular Ehlers Danlos. There were no deaths and no rare diagnosis reported more than one individual with a more severe outcome. One person with no rare health conditions exhibited a more severe outcome. When grouped by affected organ system, there was no significant association between diagnosis group and COVID-19 positivity rates, nor severe outcomes.

Conclusions: In this preliminary study, individuals in several rare disease groups experienced more severe outcomes with COVID-19. However, when divided by organ system, no groups faired significantly worse; more data are needed to fully understand the COVID X rare disease effects. Continued investigation of COVID-19 in rare disease backgrounds will inform diagnosis-specific health guidance.

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P25.013.D Effect of *TNF-α* and *CCR5* genetic variants on respiratory support in patients with COVID-19

Natalia Gorovenko[1], *Zoia Rossokha*[2], *Liliya Fishchuk*[2], *Valeriy Pokhylko*[3], *Yuliia Cherniavska*[3], *Svitlana Tsvirenko*[3], *Serhii Kovtun*[4], *Natalia Medvedieva*[2], *Victoriia Vershyhora*[2], *Ljudmila Brishevac*[1]

[1]*The Shupyk National Medical Academy of Postgraduate Education, Kyiv, Ukraine,* [2]*State Institution Reference-centre for Molecular Diagnostic of Public Health Ministry of Ukraine, Kyiv, Ukraine,* [3]*Ukrainian Medical Stomatological Academy, Poltava, Ukraine,* [4]*Poltava Regional Clinical Infectious Diseases Hospital of Poltava Regional Council, Poltava, Ukraine.*

Introduction: It is known that tumor necrosis factor alpha (TNF-α) and C-C chemokine receptor type 5 (CCR5) are involved in the various immunogenetic events, in particular, in the course of various infectious diseases. We assumed that variants of *TNF*-α and *CCR5* genes may affect on the course of COVID-19 pneumonia. The aim of our study was to analyze the effect of the *TNF-α* gene (G308A, rs1800629) and the *CCR5* gene (del32, rs333) variants on the course of severe COVID-19 pneumonia in patients.

Materials and Methods: The study group included 31 patients (16 men and 15 women) aged 58.90 ± 18.98 years with diagnosis "viral COVID-19 pneumonia" treated at the intensive care unit. 19 (61%) patients on admission to the hospital have already received oxygen therapy (using an oxygen mask). 7 (23%) patients were hospitalized for lung mechanical ventilation due to the severity of the condition and respiratory failure. Genes variants was carried out by a molecular method using PCR-RFLP and allele-specific PCR, respectively.

Results: There were found a significant correlation of the *TNF-α* gene variants (308A-allele) and duration of intubations on lung mechanical ventilation ($r = 0.967$, $p = 0.0001$). Also a significant positive correlation was between del32/allele genotypes of *CCR5* gene in patients and duration of stay on oxygen therapy using an oxygen mask ($r = 0.455$, $p = 0.044$), stay on lung mechanical ventilation ($r = 0.760$, $p = 0.047$) and the total duration of oxygen therapy ($r = 0.467$, $p = 0.029$).

Conclusions: The variants of the *TNF-α* and *CCR5* genes was the genetic predictor of increased need for respiratory support in patients with COVID-19 pneumonia.

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P25.014.A Design of a cost-effective diagnosis tool for SARS-Cov-2 variant detection through a next generation sequencing (NGS) based strategy

Mª Mercedes Montero[1], *Pau Rodríguez*[1], *Juan Ramón González*[2], *Lluís Armengol*[1], *Jairo Rodríguez*[1]

[1]*Quantitative Genomics Medicine Laboratories (qGenomics), Esplugues de Llobregat, Spain,* [2]*Barcelona Institute for Global Health (ISGlobal), Barcelona, Spain.*

Current pandemic situation together with the continuous emergence of new SARS-CoV-2 variants reveal the need of developing a more specific tool than PCR-based methods that allows both Covid-19 diagnosis and specific variant detection at a reduced cost. Thus, with the aim of providing a strategy with these characteristics arises our NGS-COVID test, a NGS based methodology that allows differential variant diagnosis with a similar cost to PCR assays.

This strategy works with RNA samples obtained from nasopharyngeal swabs which are processed with our custom protocol for sequencing with an Illumina platform. Custom pipelines for sample analysis were developed and specific viral and control human regions and variant associated mutations were detected. Experiment results showed that the strategy provides high coverage rates and demonstrates the capability to differentiate positive and negative samples with high accuracy.

Taken together, these results suggest that our test could be in the market in a very short-term period, providing a cost-effective strategy for variant diagnosis at a very reduced cost in comparison with the current sequencing methods. Having available a test with these characteristics is essential for epidemiological surveillance, providing valuable information for pandemic monitoring or for the current vaccination strategies. Lastly, the versatility of our test includes the capability of identifying the presence of other common respiratory viruses as well as the possibility of adding new variants as they emerge, making our strategy suitable for future pandemic situations.

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P25.016.C Genetic predisposition to severe COVID-19 symptoms differs by sex within the ALFA study

Natalia Vilor-Tejedor[1,2,3], ***Patricia Genius***[1,4], *Blanca Rodríguez-Fernández*[1], *Carolina Minguillon*[1,5,6], *Karine Fauria*[1,5], *Manuel Castro de Moura*[7], *David Piñeyro*[7], *Manel Esteller*[7,8,9], *Jose Luis Molinuevo*[1], *Roderic Guigo*[2,10], *Arcadi Navarro*[1,9,11], *Eider M. Arenaza-Urquijo*[1,5,6], *Juan Domingo Gispert*[1,5,10]

[1]*Barcelonaβeta Brain Research Center (BBRC), Pasqual Maragall Foundation, Barcelona, Spain,* [2]*Centre for Genomic Regulation (CRG), Barcelona Institute of Science and Technology (BIST), Barcelona, Spain,* [3]*Erasmus MC University Medical Center Rotterdam, Department of Clinical Genetics, Rotterdam, Netherlands,* [4]*University of Vic-Central University of Catalonia, Vic, Spain,* [5]*IMIM (Hospital del Mar Medical Research Institute), Barcelona, Spain,* [6]*Centro de Investigación Biomédica en Red de Fragilidad y Envejecimiento Saludable (CIBERFES), Madrid, Spain,* [7]*Josep Carreras Leukaemia Research Institute (IJC), Barcelona, Spain,* [8]*Physiological Sciences Department,*

School of Medicine and Health Sciences, University of Barcelona (UB), Barcelona, Spain, [9]Institucio Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain, [10]Pompeu Fabra University, Barcelona, Spain, [11]Institute of Evolutionary Biology (CSIC-UPF), Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona, Spain.

Introduction: Identification of genetic predisposition impacting on COVID-19's complications may hold the potential to identify common mechanisms leading to these COVID-19 complications. Our aim was to estimate whether genetic predisposition to a wide range of heritable traits was associated with the estimated genetic predisposition for the COVID-19 outcomes from the January 2021 release of the Covid19 Host Genetics Initiative.

Methods: A total of 2,280 cognitively unimpaired participants (45-74 years) from the ALFA study were included (63.3% women). Genetic predisposition for several traits (N = 33), and COVID-19 outcomes (N = 2) were calculated through polygenic risk scores. Association analyses included genetic predisposition to COVID-19 outcomes as the variables of interest. Models were adjusted for age, sex, the four principal genetic components and batch effects. Stratified models by sex were also assessed. The threshold of significance was established by approximating the total number of effective tests ($p < 0.005$).

Results: Results showed significant differences in genetic predisposition to severe COVID-19 symptoms among sexes. Higher genetic risk to larger body mass index was associated with higher genetic risk of critically ill COVID-19+ status in women. In addition, we found an association between higher genetic risk to anxiety and a protective genetic effect to SARS-CoV-2 infection.

Conclusions: We provide evidence that genetic susceptibility to some diseases was related to genetic predisposition to COVID-19 complications specifically in women.Acknowledgements: The research leading to these results has received funding from "la Caixa" Foundation (LCF/PR/GN17/10300004).

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P25.018.A Confirming rapid test diagnostics in SARS-CoV-2 infections using RT-PCR

Rodica Madalina Cristea[1,2], *Pompilia Petruta Apostol*[2], *Madalina Andreea Ivan*[1,2], *Maria Pelin*[1,2], *Nitu Florin Robert*[1,2], *Robert Adrian Nitu*[1,2], *Ortansa Csutak*[1]

[1]University of Bucharest, Faculty of Biology, Bucharest, Romania, [2]SC Clinica Sante SRL, Bucharest, Romania.

Introduction: Discovered in 2019, the novel Coronavirus can infect human and causes acute respiratory syndrome (SARS-CoV-2) infection has spread to more than 200 countries, causing thousands of deaths. Therefore, a huge need for rapidly scale-up testing services became essential for an effective treatment and control the virus spread. Rapid Diagnostic Tests (RDTs) are equipment-free, can be used by minimally trained healthcare workers generating rapid results. Aim: Using RT-PCR methodology in order to verify the accuracy of some Ag RDTs.

Materials and Methods: From about 63.000 samples tested in our laboratory until now, our study focused on 31 patients from a prison tested positive by Ag RDTs. All patients were men aged between 19-71. Samples were represented by nasopharyngeal and oropharyngeal specimens. COVID-19 diagnosis entails direct detection of SARS-CoV-2 RNA by nucleic acid amplification technology.

Results: The results obtained by RDTs were confirmed by RT-PCR method, for all patients. Thus, false-positive or false-negative results were avoided. Weak-positive results obtained with RDTs were confirmed by a Ct value >26, which means a small viral amount in the analysed samples.

Conclusions: The Ag RDTs proved to be reliable, all results obtained being confirmed by RT-PCR. Rapid diagnostic tests indicate the infection in the acute phase of the disease, the infection in asymptomatic people, and whether the infection is active, offer results in less than 30 minutes and are easy to apply. Choosing a kit which offer the most reliable, rapid and accurate diagnostic for SARS-CoV-2 infection its very important this pandemic time.

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P25.019.B Transgenic cell lines in coronavirus research

Alexander A. Dolskiy, Sergei A. Bodnev, Anastasia A. Nazarenko, Anastasia M. Smirnova, Irina V. Grishchenko, Tatiana V. Tregubchak, Ilnaz R. Imatdinov, Oleg V. Pyankov, Elena V. Gavrilova, Rinat A. Maksyutov, Dmitry V. Yudkin, ***Anna K. Matveeva***

State Research Center of Virology and Biotechnology "Vector", Rospotrebnadzor, World-Class Genomic Research Center for Biological Safety and Technological Independence, Federal Scientific and Technical Program on the Development of Genetic Technologies, Koltsovo, Novosibirsk Oblast, Russian Federation.

Introduction: SARS-CoV and SARS-CoV-2 are coronaviruses that have caused massive epidemics. The SARS-CoV-2 pandemic is still ongoing, it has affected millions of people worldwide and caused more than 2 million deaths. We obtained Vero-based cell lines with a deletion of the cytoplasmic N-tail (CT) and transmembrane (TM) domains of the BST2 gene (Vero-BST2Δ221) and with LAMP1 overexpression (Vero.Lu3 and Vero.Lu5), and we investigated the production of coronaviruses in these cell lines.

Materials and methods: A partial homozygous deletion of the TM and CT domains of the BST2 gene was obtained using CRISPR/Cas9. LAMP1-overexpressing cell lines were obtained through LAMP1 transgenic integration into the Vero genome based on the Sleeping Beauty transposon system. Each manipulation with live SARS-CoV (Urbani, Erasmus University Medical Center, Rotterdam) and SARS-CoV-2 (nCoV/Victoria/1/2020) viruses was performed under BSL-3 conditions.

Results: We showed that SARS-CoV and SARS-CoV-2 production substantially decreased in cells with the CT and TM deletion of the BST2 gene, while LAMP1-overexpressing cells exhibited increased production of both viruses. Cells were infected with viruses in the presence and absence of TPCK-trypsin, and a significant impact on viral production was not observed.

Conclusion: The TM of BST2 is targeted by viral proteins, thereby reducing its antiviral activity. Deletion of TM resulted in suppression of viral production due to BST2 activation. LAMP1 promotes virus particle maturation by promoting sufficient pH levels in lysosomes; therefore, LAMP1 overexpression increases viral release.This work was supported by the Ministry of Science and Higher Education of the Russian Federation (agreement # 075-15-2019-1665).

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P25.021.D Full-genome sequences of the first nine SARS-CoV-2 viruses from Azerbaijan

AGHARZA AGHAYEV, *Valeh Huseynov, Elin Aliyev, Ramin Bayramlı*

National Hematology and Transfusiology Center, Department of Medical Genetics, Baku, Azerbaijan.

Introduction: COVID-19, caused by the novel SARS-CoV-2 virus, started in China in late 2019 and soon became a pandemic outbreak. Robust surveillance mechanisms should be implemented to control the ongoing pandemic such as rapid and scalable detection of infection, strain evolution interrogation, and novel biomarker identification. Whole-genome sequencing enables the identification of the origins which makes it possible to track the viral evolution. To gain further understanding of the molecular epidemiology of the outbreak in Azerbaijan, a full-genome sequencing was performed on nine SARS-CoV-2 isolates.

Material and methods: Shotgun transcriptome sequencing was performed using RNA extracted from nasopharyngeal swabs of SARS-CoV-2 positive patients. Multiple sequence alignment and phylogenetic tools were utilized to compare the assembled SARS-CoV-2 genomes to publicly available sequences.

Results: Sequenced samples from Azerbaijan fall into GR/20B clade which is associated with European lineages. 8 out of 9 sequences fall into a basal clade which is currently observed only in Azerbaijan. However, due to undersampling among the neighboring countries, this lineage may be circulating more widely. Only 1 sequence shared similarities with the genome published by Turkey. One viral strain presented a two previously unreported mutation in the ORF14 and nsp3 region, namely p. G50N and p.N1587Y.

Conclusions: SARS-CoV-2 whole-genome sequencing is a highly feasible and powerful approach for tracking virus transmission. Genomic data can be used to determine the most appropriate public health decisions to control the pandemic. Epidemiologically-defined clusters displayed specific mutations, suggesting molecular signatures for strains coming from areas that were isolated during the lockdown.

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