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Abstracts of the ECTS Congress 2021
48th European Calcified Tissue Society Congress
ECTS 2021 Digital Congress
6 – 8 May 2021
followed by the ECTS@Home from 19 – 20 May,
10 – 11 June and on 18 June 2021

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Plenary Oral Presentations

Plenary Oral Presentations 1: Genetics & Bone

PLO01

Novel BMD loci identified by whole genome sequencing and CRISPR editing in zebrafish: The NHLBI Trans-Omics for Precision Medicine (TOPMED) study

Yi-Hsiang Hsu[a,b], Joyce W. Tang[c], Hanfei Xu[d], Cecily Choy[c], May Montasser[e], Carolyn Crandall[f], Ming-Ju Tsai[a], Mao Fu[e], Jeff R. O'Connell[e], James Perry[e], Shabnam Salimi[e], Elizabeth Streeten[e], Charles Farber[g], David Karasik[h], David M. Evans[i], Jonathan H. Tobias[j], Brent Richards[k], Ching-Ti Liu[d], Robert Wallace[l], John Blangero[m], Kerri Wiggins[n], Rebecca Jackson[o], Douglas Kiel[a], Braxton D. Mitchell[e], Ronald Y. Kwon[c]

[a]*Harvard Medical School, HSL Marcus Institute for Aging Research, Boston, United States*
[b]*Broad Institute of MIT and Harvard, Medical and Population Genetics Research, Cambridge, United States*
[c]*Univ. of Washington, Orthopaedics and Sports Medicine, Seattle, United States*
[d]*Boston Univ., School of Public Health, Boston, United States*
[e]*University of Maryland, School of Medicine, Baltimore, United States*
[f]*University of California, School of Medicine, Los Angeles, United States*
[g]*University of Virginia, School of Public Health, Charlottesville, United States*
[h]*Hebrew SeniorLife Marcus Institute for Aging Research, musculoskeletal Center, Boston, United States*
[i]*University of Queensland, Diamantina Institute, Woolloongabba, Australia*
[j]*University of Bristol, Department of Clinical Science at North Bristol, Bristol, United Kingdom*
[k]*McGill University, Lady Davis Institute- Jewish General Hospital, Montréal, Canada*
[l]*University of Iowa, School of Medicine, Iowa City, United States*
[m]*University of Texas, Rio Grande Valley School of Medicine, Edinburg, United States*
[n]*Univ. of Washington, School of Public Health, Seattle, United States*
[o]*Ohio State University, Wexner Medical Center, Columbus, United States*

Background/Introduction: The discovery of causal genetic variants creates opportunities for new disease diagnostics and therapies. Whole-genome sequencing (WGS) is rapidly being incorporated into mainstream biomedical research to provide comprehensive enumeration of sequence variation.

Purpose: To identify novel rare ding variants that are associated with BMD.

Methods: We utilized WGS (average 30X coverage) in ~10K Caucasians from the NHLBI TOPMED Program and UK10K project (ALSPAC, TWINSUK ~10X coverage). We performed gene-based collapsing association tests to identify rare functional coding variants (MAF ≤ 0.5%) associated with BMD adjusting for age, age², sex, weight, height, ancestral genetic background, cohort, and menopausal status (women). Only loss-of-function (LoF), protein-altering short INDEL and missense variants with deleterious effects were included. CRISPR-Cas9 zebrafish mutants were generated.

Results: The genome-wide significance p-value is set as 4.27x10E-6 after Bonferroni correction. The most significant association was found in the *IGHE* gene (p-value=7.18x10E-8) with lower LS BMD. The other novel findings included *SLC26A11, ERGIC3* and *STMN1* with LSBMD and *TFAP2E, CYP2B6, GDF10* and *IL6* with FNBMD. GDF10 (growth differentiation factor 10) is a member of the transforming growth factor ß superfamily. Among associated functional coding variants, SNP P468S is located in the mature region downstream of the RXXR cleavage site and suppress *GDF10* function. To address the involvement of *GDF10* in bone metabolism, we generated zebrafish mutants for gdf10a and gdf10b and analyzed them using microCT-based spinal phenomics (160 measures/animal). We administered CRISPR-Cas9:gRNA complexes targeting three sites on gdf10a or gdf10b. Sequencing analysis revealed that this produced bi-allelic out-of-frame mutations with an efficiency of ~90% for each gene. During early development (12 days), somatic mutants for both gdf10a and gdf10b exhibited increased vertebral ossification. As adults (3mo), somatic mutants for gdf10a exhibited reduced vertebral bone mass and mineralization ($p<0.005$).

Conclusion(s): Our studies support a function for *GDF10* in the development and growth of the skeleton.

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PLO02

Development of a disease model for autosomal recessive osteopetrosis and CRISPR/Cas9-based gene therapeutic approaches in human induced pluripotent stem cells

Floriane Hennig[a], Uta Rössler[b], Nina Stelzer[b], Johannes Kopp[b], Kent Søe[c], Harald Stachelscheid[d], Uwe Kornak[a]

[a]*University Medical Center Göttingen, Institute of Human Genetics, Göttingen, Germany*
[b]*Charité - Universitätsmedizin Berlin, Institute for Medical Genetics and Human Genetics, Berlin, Germany*
[c]*Odense University Hospital, Clinical Cell Biology- Department of Pathology, Odense, Denmark*
[d]*Charité - Universitätsmedizin Berlin- Berlin Institute of Health, Core Facility Stem Cells, Berlin, Germany*

Background/Introduction: Osteoclasts differentiate from hematopoietic stem cells and are the only bone resorbing cell type. Mutations affecting the development or function of osteoclasts can cause severe bone diseases like autosomal recessive osteopetrosis (ARO). Autologous transplantation of gene-corrected cells could be a future treatment for ARO. Since patient-derived primary cells are scarce, we wanted to evaluate the potential of human induced pluripotent stem cells (hiPSCs) for disease modelling and optimization of therapeutic approaches.

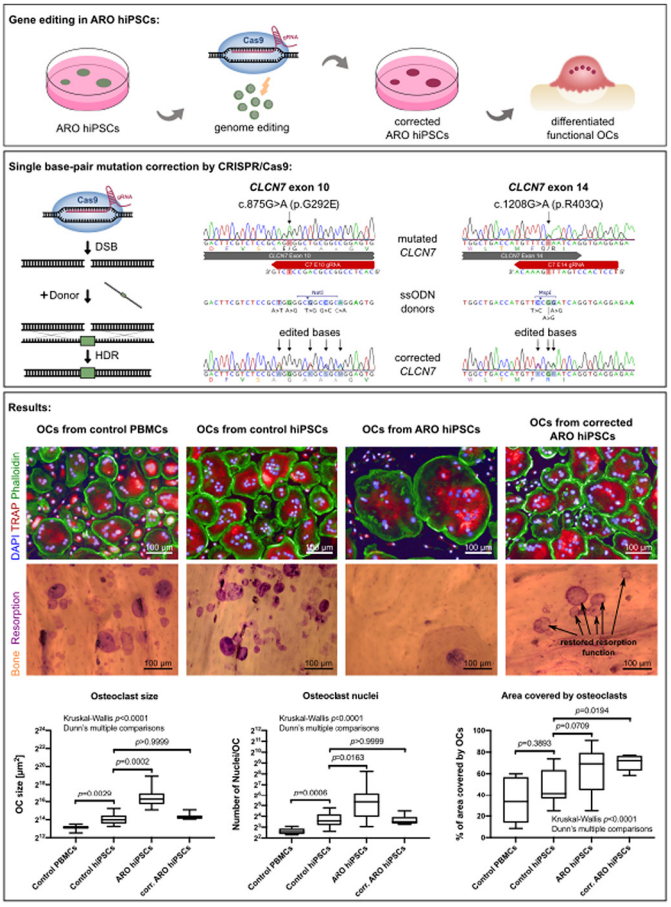
Purpose: For testing gene therapeutic approaches for ARO, we established a hiPSC-based disease model.

Methods: We generated hiPSCs from an ARO patient carrying compound heterozygous *CLCN7* mutations and performed CRISPR/Cas9-based single base-pair editing with Cas9 ribonucleoproteins and single-stranded oligodeoxynucleotide donors for mutation correction. hiPSCs were differentiated into osteoclasts using an optimized protocol and were analysed functionally.

Results: When differentiating hiPSCs from healthy donors, we were able to generate osteoclasts showing cell morphology, expression patterns, and bone resorption activity similar to PBMC-derived osteoclasts. Osteoclasts differentiated from our ARO patient hiPSC line were larger and not able to resorb bone, thus recapitulating the cellular ARO phenotype. After mutation correction, a normalization of resorption activity and cell size was observed in ARO hiPSC-derived osteoclasts. Detailed analysis of the resorption activity is ongoing. Whole genome sequencing did not reveal relevant off-target effects.

Conclusion(s): We demonstrated that osteoclasts differentiated from ARO hiPSCs can be used as a disease and therapy model for ARO. Genome editing rescues osteoclast function, providing a proof-of-concept for a CRISPR/Cas9-based gene therapy for ARO.

doi:10.1016/j.bonr.2021.100766



PLO03

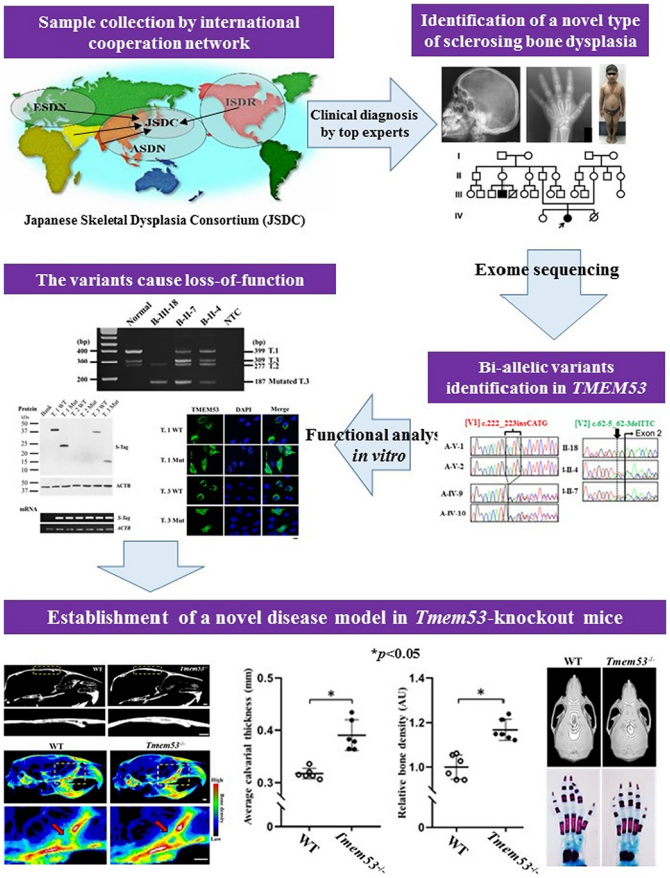
Bi-allelic loss-of-function variants in TMEM53 cause a novel type of sclerosing bone disorder in human and mouse

Long Guo, Jingyi Xue, Zheng Wang, Shiro Ikegawa
RIKEN Center for Integrative Medical Sciences, Laboratory for Bone and Joint Diseases, Tokyo, Japan

Background/Introduction: Sclerosing bone disorder (SBD) is a heterogeneous group of monogenic diseases characterized by increased bone density. More than 40 disease entities, including osteopetrosis, dysosteosclerosis, and sclerosteosis, fall into this category. Identification of dozens of the causal genes for SBD over the past few decades has not only improved precise diagnosis and genetic counseling of the diseases but also continuously driven the research of bone formation and development, which led to the therapeutic innovation of bone density-related diseases.

Purpose: To identify new disease genes for SBD.

Methods: We recruited patients with unknown types of SBD by the Japanese Skeletal Dysplasia Consortium. The causal variants were identified by whole exome sequencing. The function of these variants was analyzed using RT-PCR, Western blot and immunofluorescence staining. The corresponding gene was disrupted in mice by CRISPR/Cas9 system. Phenotypic comparison between the patients and the knockout mice was performed by microCT, X-ray, and skeletal preparation.



Results: We discovered a novel type of SBD in five patients from four independent families, which is characterized by hyperostosis of the calvaria and the skull base, mild platyspondyly, wide pubis and ischia, broadening of the femoral neck, and mild shortening and diaphyseal broadening of the short tubular bones. The disorder is caused by bi-allelic loss-of-function variants in *TMEM53*, which encodes nuclear envelope transmembrane (NET) protein 53 (TMEM53, also known as NET4). The *Tmem53*$^{-/-}$ mice generated in this study recapitulated the patients' skeletal phenotype.

Conclusion(s): Our results establish a novel SBD entity in human and the corresponding disease model in mice.

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PLO04

INZ-701, a recombinant ENPP1-Fc protein, prevents ectopic mineralization in a mouse model of pseudoxanthoma elasticum

Zhiliang Cheng[a], Joely D. Jacobs[b], Kevin O'Brien[a], David Thompson[a], Jouni Uitto[b], Qiaoli Li[b], Yves Sabbagh[a]
[a]*INOZYME PHARMA, Research, Boston, United States*
[b]*Thomas Jefferson University, Dermatology and Cutaneous Biology, Philadelphia, United States*

Background/Introduction: Pseudoxanthoma elasticum (PXE) is an autosomal recessive disease characterized by ectopic mineralization of the skin, eyes and the cardiovascular system. PXE is predominantly caused by bi-allelic inactivating mutations in *ABCC6* encoding a putative efflux hepatic transporter ABCC6. Recent studies demonstrated that the absence of ABCC6-dependent adenosine triphosphate (ATP) release from the liver and consequently reduced plasma inorganic pyrophosphate (PPi) levels are critical pathogenic features of PXE.

Purpose: This study examined whether treatment with recombinant ENPP1, the principal enzyme that generates extracellular PPi from ATP, could restore plasma PPi levels, a potent mineralization inhibitor, and prevent ectopic mineralization in ABCC6 deficiency.

Methods: INZ-701, a recombinant human ENPP1-Fc fusion protein that is being developed as an enzyme replacement therapy for the treatment of ENPP1 deficiency, was tested for prevention of ectopic mineralization in an *Abcc6*$^{-/-}$ mouse model of PXE. *Abcc6*$^{-/-}$ mice, at 5-6 weeks of age, the time of earliest stages of ectopic mineralization, were treated with subcutaneous injection of INZ-701 at doses of 2 and 10 mg/kg every other day for 2 or 8 weeks.

Results: Administration of INZ-701 showed a dose-dependent increase in plasma ENPP1 activity and plasma PPi level both 2 and 8 weeks after initiation of treatment. Histopathologic examination of von Kossa stained sections from vehicle-treated *Abcc6*$^{-/-}$ mice revealed extensive mineralization in the muzzle skin containing vibrissae, a biomarker of the mineralization process in these mice, while significantly reduced mineralization was detected in mice treated with INZ-701. Quantitative calcium assay demonstrated that the amount of calcium in the muzzle skin biopsies were reduced by 68% and 74% in mice administered with INZ-701 at 2 and 10 mg/kg, respectively (P values < 0.01).

Conclusion(s): These results suggest that INZ-701 might provide a promising treatment strategy for PXE, a disease with high unmet need and no approved treatment.

doi:10.1016/j.bonr.2021.100768

PLO05

Fracture healing of the mandible is impaired in a Fgfr3N534K/+ mouse model of hypochondroplasia

Anne Morice, Loisay Lea, Laurence Legeai Mallet
Imagine Institute, Laboratory of Molecular and Physiopathological Bases of Osteochondrodysplasia- INSERM UMR 1163, Paris, France

Background/Introduction: Hypochondroplasia (HCH) is the less severe form of chondrodysplasia due to Fibroblast Growth Factor Receptor 3 (*FGFR3)* gain-of-function mutations. HCH patients are characterized by short limb, accentuated lordosis, large head and prognathism. Our team generated the first *Hch* mouse model (*Fgfr3*$^{N534K/+}$) expressing the most common missense mutation (p. Asn540Lys). This *Hch* mouse model mimics human pathology, the endochondral and membranous ossification are impaired as observed in axial, appendicular and craniofacial skeleton.

Purpose: Craniofacial bone healing has never been studied in *Hch* mouse model. Here, we aimed to analyse bone repair following a non-stabilized mandibular fracture in *Fgfr3*$^{N534K/+}$ mice and its control littermates.

Methods: The fractures were performed at 6 weeks of age on the right mandible in controls and mutants. Morphometric analysis based on CT-scans and histomorphometric studies were performed at several key points of fracture repair.

Results: Lower values of BV/TV (Bone Volume/Tissue Volume) were observed in the calluses of the fractured mandibles in *Hch* mice compared to controls at day 14 (-8,842±2,218 %; $p < 0.001$) and at day 28 post-injury (-4,352±1,303 %; p=0.003). Callus volume was significantly increased at day 14 in *Fgfr3*$^{N534K/+}$ mice (+55,75±20,97 %; p=0,01) compared to control. Histomorphometric analyses, using alcian blue/picrosirus staining of the calluses, confirmed the involvement of endochondral ossification process in bone healing. Cartilage resorption was effective at day 21 in all control mice (n=5), whereas in 4/6 mutants the cartilage was present. In the calluses, intertrabecular area was significantly increased in mutant mice compared to controls at day 14, these data were confirmed by Collagen I immunostaining of the calluses (+18,98± 6,004 %; p=0.01).

Conclusion(s): These data highlighted delayed cartilage resorption and impairment of chondrocyte differentiation and osteoblast differentiation in *Fgfr3N534K/+* mice during fracture healing of the mandible. All these data will improve our understanding of the hypochondroplastic bone features during bone repair.

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PLO06

Knockout of osteopontin or bone sialoprotein induces opposite response to mechanical stimulation

Mathieu Maalouf[a], Hawa Cinar[a], Wafa Bouleftour-Esquis[b], Arnaud Vanden-Boscche[a], Mireille Thomas[a], Norbert Laroche[a], Laurence Vico[a], Alain Guignandon[a], Luc Malaval[a]
[a]*INSERM - Université Jean Monnet, U1059-Sainbiose- LBTO, St Priest en Jarez, France*
[b]*Université Jean Monnet - Département Universitaire de la Recherche et de l'Enseignement, Institut de Cancérologie Lucien Neuwirth, Saint-Etienne, France*

Background/Introduction: Bone sialoprotein (BSP) and osteopontin (OPN), co-expressed by bone cells, display rapid gene response to mechanical signals.

Purpose: To clarify their roles in this process, 2 months old male wild type, OPN knockout (OPN$^{-/-}$), BSP$^{-/-}$ and OPN/BSP double knockout (DKO) mice (housed in CEEAL-UJM agreement Nr98) were distributed in control (CT) and vibrated (VB) groups (n=10/group).

Methods: Mice stayed on a vibrating plate (2g/45Hz), 15 min/day for 6 weeks (plate kept still for CT). Bones were collected for micro-tomography, histomorphometry, RT-qPCR.

Results: Vibration reduced trabecular volume in OPN$^{-/-}$ mice (vertebra BV/TV: -18%, $p<0.05$) with increased osteoclast surfaces (Oc.S/BS: +72%, $p<0.01$). OPN$^{-/-}$ lost femoral cortical bone (Ct.Th: -9%, $p<0.01$) under increased resorption (Ec.Oc.S/B.S: +95%, $p<0.05$). Strikingly, VB BSP$^{-/-}$ mice gained cortical bone (Ct.Th: +7%, $p<0.01$), with significant increase in formation, less osteoclasts (Ps.Oc.S/B.S: -68%, $p<0.05$) and lower RANKL and CathK expression. Sequential fluorochrome labeling (Figure 1) documented increased formation in VB BSP$^{-/-}$ cortex (Figure 2). No effect was observed in other genotypes.

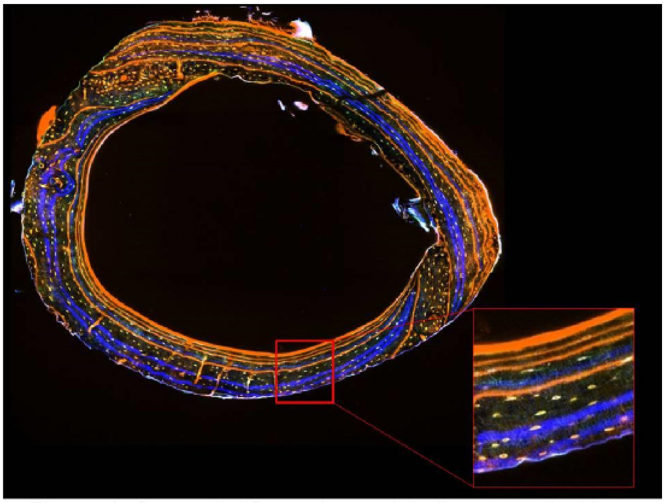

Figure 1: Sequential fluorochrome labeling on cortical bone using alizarin complexone (orange) and calcein blue (blue). VB BSP$^{-/-}$ mouse.

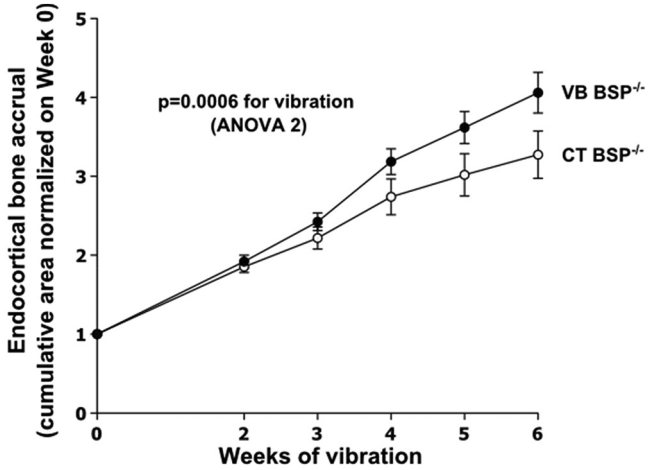

Figure 2: Endocortical bone accrual in CT (empty circles) and VB (black circles) BSP$^{-/-}$ mice. Values are mean ± SEM of 6 mice.

Conclusion(s): BSP and OPN thus play crucial and opposite roles in bone response to mechanical stimulation. Because BSP-/- mice overexpress OPN, and DKO mice showed no effect, interaction between these two factors seems required for a proper skeletal response to mechanical challenges.

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Plenary Oral Presentations 2: Novel Aspects of Osteoporosis and Treatments

PLO07

A 20-year comparison of sex-specific fracture incidence between Type 1 and Type 2 diabetics and non-diabetics in Denmark

Mohamad Nasser[a], Annika Kvist[b], Morten Frost[a], Peter Vestergaard[c,d,e], Andrea M. Burden[b]

[a]*University of Southern Denmark, Department of Endocrinology and Metabolism- Molecular Endocrinology & Stem Cell Research Unit KMEB Odense University Hospital, Odense, Denmark*

[b]*Eth Zurich, Department of Chemistry and Applied Biosciences, Zurich, Switzerland*

[c]*Steno Diabetes Centre, North Jutland, Aalborg, Denmark*

[d]*Aalborg University, Department of Endocrinology, Aalborg, Denmark*

[e]*Aalborg University, Department of Medicine, Aalborg, Denmark*

Background/Introduction: Major osteoporotic fractures have declined in Nordic and western countries over the last two decades, despite an aging society of the general population. However, little is known if the decreasing trend of fractures is similar in patients with diabetes.

Purpose: To investigate the incidence rate (IR) of fractures in adult (age 18+) patients in Denmark between 1997-2017 with Type 1 Diabetes (T1D) and Type 2 Diabetes (T2D) compared to non-diabetics, stratified by sex.

Methods: All patients aged 18+ with a bone fracture (excluding skull and facial) between 1997 and 2017 were identified from the Danish National Health Service Register and linked to the Danish Medicines Agency Register of Medicinal Products Statistics (RMPS). Gender-specific IRs of fractures per 10,000 person years (PYs) were estimated. All analyses were stratified by diabetes diagnosis, defined as T1D, T2D, and non-diabetic.

Results: Overall, we identified a 25% decline in the IRs of fractures among T1D (from 571.6 to 427.0), a 58% decline among T2D (from 840.1 to 250.6), and a 10% decline among non-diabetics (from 179.2 to 161.5), between 1997 and 2017, respectively. Among males, the IRs declined 35% among T1D (from 499.3 to 322.9), 67% among T2D (from 786.9 to 263.8), and 10% among non-diabetics (from 191.8 to 186.9). Similarly, among women, we observed a 12% decline among T1D (from 666.3 to 586.3), a 49% decline among T2D (from 896.0 to 455.5), and 3% in non-diabetics (from 191.8 to 186.9).

Conclusion(s): In this 20-year population-based observation period, we identified a declining trend of fractures among diabetic patients, particularly among those with T2D. However, despite decreasing incidence, the IR of fractures in 2017 among patients with T1D or T2D was substantially higher than non-diabetics in Denmark, particularly among women, thereby highlighting the need to improve fracture management in these patients.

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PLO08

Risk of fracture in patients with glucocorticoid requiring diseases

Giovanni Adami, Davide Gatti, Angelo Fassio, Alessandro Giollo, Ombretta Viapiana, Maurizio Rossini

University of Verona, Rheumatology Unit, Verona, Italy

Background/Introduction: Glucocorticoid-Induced Osteoporosis (GIOP) is the most common form of secondary osteoporosis. Nevertheless, the independent role of GCs and GC requiring diseases on fracture risk is still unclear.

Purpose: The aim of the present work is to assess the fracture risk associated with several GC requiring diseases.

Methods: We conducted a retrospective analysis of a nation-wide cohort. Many comorbidities were included (rheumatoid arthritis, psoriatic arthritis, UCTD, SLE, systemic sclerosis, COPD, multiple sclerosis, IBD, severe physical handicap, diabetes, Parkinson's and HIV). We generate groups of patients age and T-score matched via propensity score matching.

Results: 59950 women were included in the analysis. Among 13,546 women with comorbidity 3114 (23.0%) had diabetes; 3008 (22.2%) rheumatoid arthritis; 1910 (14.1%) UCTD; 1614 (11,9%) COPD; 942 (7,0%) IBD and 900 (6.8%) neurological diseases. Glucocorticoid intake ≥5 mg/day for ≥3 months (after 1:1 matching by age and T-scores) was significantly associated with vertebral fractures (aOR 1.5 95% CI 1.3-1.7) but not with non-femoral non-vertebral fractures (aOR 1.0 95% CI 0.9-1.2).

Figure 1 and **Figure 2** show the ORs for vertebral or hip fractures and non-vertebral or non-hip fractures. Diseases with increased risk of fracture, independently from glucocorticoid intake, were rheumatoid arthritis, COPD and neurological diseases for non-vertebral and non-hip fractures and COPD and neurological diseases for vertebral or hip fractures.

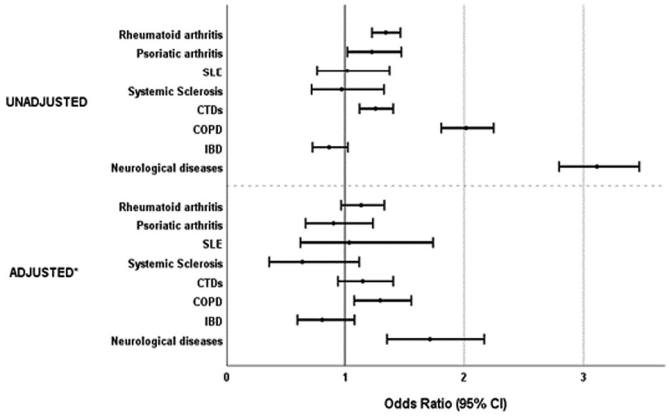

Figure 1. Forest plot showing the risk of vertebral or hip fractures in different diseases (adjusted for *age, bone mineral density, menopausal status, glucocorticoid intake and familiar history of fragility fractures)*

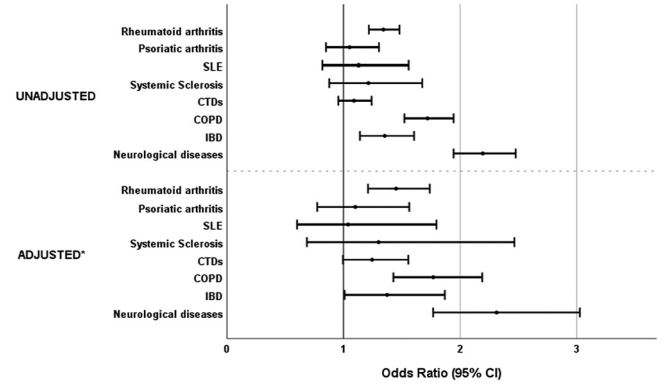

Figure 2. Forest plot showing the risk of non-vertebral and non-hip fractures in different diseases. (adjusted for *age, bone mineral density, menopausal status, glucocorticoid intake and familiar history of fragility fractures)*

Conclusion(s): Rheumatoid arthritis, COPD and neruological diseases were independently associated with an increased risk of non-vertebral and non-hip fractures whereas only COPD and neurological diseases were associated with vertebral or hip fractures.

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PLO09

Week 26 results from the PaTH Forward Open-Label Extension Trial Support TransCon PTH as a potential hormone replacement therapy for patients with hypoparathyroidism

Lars Rejnmark[a], Peter Schwarz[b], Mishaela Rubin[c], Tamara Vokes[d], Bart Clarke[e], Intekhab Ahmed[f], Andrea Palermo[g], Claudio Marcocci[h], Uberto Pagotto[i], Erik Eriksen[j], Sanchita Mourya[k], Denka Markova[l], Susanne Pihl[m], Michael Beckert[n], Aimee Shu[k], Aliya Khan[o]

[a]*Aarhus University Hospital, Department of Clinical Medicine - The Department of Endocrinology and Diabetes, Aarhus, Denmark*
[b]*Rigshospitalet, Department of Endocrinology, Copenhagen, Denmark*
[c]*Columbia University, Department of Endocrinology, New York, United States*
[d]*University of Chicago, Osteoporosis and Metabolic Bone Disease Clinic, Chicago, United States*
[e]*Mayo Clinic, Endocrinology, Rochester, United States*
[f]*Thomas Jefferson University, Endocrinology- Diabetes & Metabolic Diseases, Philadelphia, United States*
[g]*Universita Campus Bio-Medico, Endocrinology and Diabetes, Rome, Italy*
[h]*University of Pisa, Clinical and Experimental Medicine, Pisa, Italy*
[i]*School of Medicine and Surgery- Alma Mater Studiorum University of Bologna- Policlinic S.Orsola, Department of Medical and Surgical Sciences, Bologna, Italy*
[j]*Oslo University Hospital- Institue of Clinical Medicine, Department of Endocrinology- Morbid Obesity and Preventive Medicine, Oslo, Norway*
[k]*Ascendis Pharma- Inc., Clinical Development, Palo Alto, United States*
[l]*Ascendis Pharma- Inc., Biostatistics, Palo Alto, United States*
[m]*Ascendis Pharma A/S, Bioanalysis & PK/PD, Hellerup, Denmark*
[n]*Ascendis Pharma A/S, Clinical Development, Hellerup, Denmark*
[o]*Bone Research & Education Centre, Bone Research & Education Centre, Oakville, Canada*

Background/Introduction: TransCon PTH is an investigational long-acting prodrug of PTH(1-34) for the treatment of hypoparathyroidism.

Purpose: Week 26 results are reported from the phase 2 PaTH Forward Open-Label Extension (OLE) Trial evaluating TransCon PTH in adults with hypoparathyroidism treated with standard of care (SoC; active vitamin D and calcium).

Methods: Subjects received fixed doses of TransCon PTH 15, 18, or 21 µg PTH(1-34)/day or placebo for 4 weeks, followed by an OLE period during which TransCon PTH dose was titrated (6–30 µg PTH[1-34]/day) with the goal to maintain normocalcemia. Efficacy end points evaluated at Week 26 included intake of active vitamin D and calcium supplements, 24-hour uCa, sCa, sP, and CaxP. Quality of life (QoL) was assessed by SF-36 and Hypoparathyroidism Patient Experience Scales (HPES).

Results: All 59 subjects completed the initial 4-week period and continued in the OLE. TransCon PTH enabled independence from SoC in most subjects by Week 26 (Table). Mean 24-hour uCa decreased from a baseline mean of 415 mg/24h to 178 mg/24h by Week 26 (n=44) while maintaining sCa and reducing sP and CaxP to fall within the normal range. SF-36 and HPES scores continued to improve through Week 26 for TransCon PTH subjects and placebo subjects switching to TransCon PTH. TransCon PTH continued to be well-tolerated with no treatment-related serious or severe adverse events.

End Points at Week 26	TransCon PTH(N=59)
Independence from SoC (no active vitamin D and Ca ≤500 mg/day)	91%
Independence from all supplements (no active vitamin D or Ca)	76%

Conclusion(s): Week 26 results from the PaTH Forward OLE demonstrated that TransCon PTH continued to enable independence from active vitamin D and calcium supplements for most subjects while maintaining normal sCa, sP, uCa, and demonstrating enhanced QoL. This supports TransCon PTH as a potential hormone replacement therapy for adults with hypoparathyroidism.

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PLO10

Adipose lipolysis is required for PTH-induced bone formation

Nathalie Alekos, Priyanka Kushwaha, Soohyun Kim, Ryan Riddle
Johns Hopkins School of Medicine, Orthopaedic Surgery, Baltimore, United States

Background/Introduction: Lipolysis of triglycerides in adipose and release of fatty acids into the circulation provides an energy source for distant tissues. Previous work from our lab indicates mitochondrial oxidation of fatty acids increases during osteoblast differentiation and is necessary for normal bone formation.

Purpose: The goal of this study was to determine if the osteo-anabolic effect of intermittent parathyroid hormone (iPTH) treatment requires fatty acid oxidation by osteoblasts and to determine if adipocyte-derived fatty acids are essential for the anabolic effect.

Methods: Mice lacking the PTH receptor in adipocytes ($Pth1r^{flox/flox}$; AdipoQ-Cre), with impaired lipolysis due to the ablation of Atgl in adipocytes ($Atgl^{flox/flox}$; AdipoQ-Cre), or mice with impaired ß-oxidation in osteoblasts ($Cpt2^{flox/flox}$; Ocn-Cre) received saline or iPTH (100 ug/kg) for six weeks. Bone architecture and histomorphometry were assessed according to standard techniques. Animal procedures were approved by the local animal care and use committee.

Results: Acute PTH treatment induces a rapid increase in serum fatty acid levels in wild-type mice, but not those lacking Pth1r or Atgl in adipocytes. In turn, ablation of Pth1r and Atgl in adipocytes, but not osteoblasts, abolished the increase in bone volume after iPTH (Table I). Consistent with the notion that fatty acids are then used by osteoblasts to fuel bone formation, the ablation of Cpt2 in osteoblasts prevented iPTH-induced bone formation.

Table 1:

	% Δ in Serum FA		BV/TV in Distal Femur			
Mouse line	WT	KO	WT-Saline	WT-PTH	KO-Saline	KO-PTH
$Pth1r^{flox/flox}$; AdipoQ-Cre	+60.24	+12.35	12.38 ± 1.08	45.59 ± 4.57*	13.51 ± 1.92	16.55 ± 1.65#
$Atgl^{flox/flox}$; AdipoQ-Cre	+55.56	+11.85	12.10 ± 1.32	36.76 ± 4.38*	12.04 ± 2.31	17.00 ± 4.37 #
$Cpt2^{flox/flox}$; Ocn-Cre	N/A	N/A	12.83 ± 1.12	20.25 ± 2.97*	12.09 ± 1.61	10.99 ± 1.51#

Values are shown as mean ± SEM, * p <0.05 vs Saline-treated control, # p <0.05 vs PTH-treated control

Conclusion(s): Collectively, these data indicate that production and utilization of adipocyte-derived fatty acids are required for iPTH to increase bone mass.

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PLO11

Reactive oxygen species lead to age-related bone loss by accelerating senescence of osteoblasts in mice

Jan-Moritz Ramge[a], Chen Xiangxu[a], Verena Fischer[a], Melanie Haffner-Luntzer[a], Jan Tuckermann[b], Karin Scharfetter-Kochanek[c], Anita Ignatius[a], Astrid Liedert[a]
[a]*Institute of Orthopeadic Research and Biomechanics, University Hospital Ulm, Ulm, Germany*
[b]*Institute of Comparative Molecular Endocrinology, University Ulm, Ulm, Germany*
[c]*Institute of Dermatology and Allergic Diseases, University Hospital Ulm, Ulm, Germany*

Background/Introduction: Reactive oxygen species (ROS) are mainly generated in mitochondria during cellular respiration and are eliminated by antioxidant defense mechanisms, including the mitochondrial enzyme superoxide dismutase (SOD)2. It has been shown that the activity of SOD2 in human osteoprogenitor cells decreased with donor age.

Purpose: In the present study, we established a mouse model of osteoblast-specific Sod2 deficiency to investigate the influence of increased ROS generation on bone mass in mice.

Methods: Female $Sod2^{fl/fl}$ and $Runx2CreSod2^{fl/fl}$ mice were sacrificed after 12 and 52 weeks, respectively. Their skeletal phenotype was analysed by µCT and histomorphometrically. Dihydroethidium was used to analyse ROS generation in bone cryosections and osteoblasts isolated from long bones. Proliferation rate and differentiation capacity of osteoblasts were assessed by BrdU assay and cytochemical stainings. Gene expression analysis was performed by qRT-PCR. Senescence-associated beta-galactosidase staining was used to detect senescent osteoblasts *in situ* and *in vitro*. Senescent-associated markers were assessed by immunohistochemical stainings in femur paraffin sections. n=6-8 per group.

Results: $Runx2CreSod2^{fl/fl}$ mice showed significantly decreased trabecular bone volume fraction, reduced trabecular number, increased trabecular separation and reduced cortical thickness in femurs compared with $Sod2^{fl/fl}$ mice. Moreover, the number and activity of osteoblasts was reduced while the number and activity of osteoclasts was increased in femurs from $Runx2CreSod2^{fl/fl}$ mice (12-weeks: NOb/BPm ($Sod2^{fl/fl}$) 19.87 ± 1.16 vs. ($Runx2CreSod2^{fl/fl}$) 11.69 ± 2.82 p<0.001; 1/mm). Increased ROS were detected in bones and osteoblasts from $Runx2CreSod2^{fl/fl}$ mice. Osteoblasts showed a decreased proliferation rate and an impaired differentiation capacity. Bones and osteoblast cultures revealed higher numbers of senescent cells. Expression levels of autophagy-associated markers Ulk1 and p62 were reduced, while expression levels of senescence-associated biomarkers p21 and $p16^{INK4a}$ were increased. Also, expression of FOXO1, IL6 and TNFα was increased in $Runx2CreSod2^{fl/fl}$ mice.

Conclusion(s): Our study suggests that osteoblast-specific Sod2 deficiency caused age-related bone loss by accelerating senescence of osteoblasts.

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PLO12

Inhibition of cyclin-dependent kinase 5 (Cdk5) increases osteoblast differentiation and bone mass through the MAPK pathway

Mubashir Ahmad[a], Torsten Kroll[b], Ann-Kristin Picke[a], Sabine Vettorazzi[a], Florian Mengele[c], Dilay Yilmaz[a], Stolz Miriam[a], Naveen Kumar Tangudu[d], David Carro Vázquez[e], Johanna Pachmayr[f], Maja Vujic Spasic[a], Aspasia Ploubidou[b], Jan Tuckermann[a]
[a]*Institute of Comparative Molecular Endocrinology- Ulm University, Helmholtzstrasse 8/1, 89081, Ulm, Germany*
[b]*Leibniz Institute on Aging – Fritz Lipmann Institute, Beutenbergstrasse 11, D-07745, Jena, Germany*
[c]*Department of trauma surgery- hand surgery and orthopaedics-Klinikum Memmingen, Bismarckstrasse 23, 87700, Memmingen, Germany*

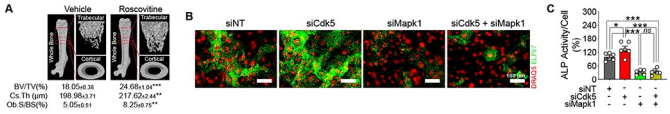
[d]UPMC Hillman Cancer Center- University of Pittsburgh, 5115 Center Avenue, 15232, Pittsburgh, United States
[e]TAmiRNA GmbH, Leberstrasse 20, 1110, Vienna, Austria
[f]Institute of Pharmacy- Paracelsus Medizinische Privatuniverstät, Strubergasse 21, 5020, Salzburg, Austria

Background/Introduction: Osteoporosis is characterized by low bone mass and altered bone microarchitecture, leading to increased risk of fractures particularly in the ageing population. Currently available low cost small molecular agents to treat osteoporosis are mainly antiresorptive and decrease the risk of fractures by stabilizing the bone mass but do not improve bone quality.

Purpose: To identify novel targets for small molecules that improve bone mass by increasing bone formation by osteoblasts is of utmost importance to improve osteoporosis therapy.

Methods: RNAi screening, Micro-computer tomography (μCT), Functional assays, Histomorphometry, Western blotting, RNA Seq.

Results: We performed an unbiased high-throughput RNAi screen in primary murine osteoblasts using alkaline phosphatase (ALP) as a readout. This functional analysis screen led to the identification of Cdk5 as a potent novel suppressor of osteoblast differentiation. *In vitro* Cdk5 siRNA knockdown or a drug inhibition with a specific inhibitor, roscovitine, enhanced ALP activity, 3.7- and 1.6-fold, respectively. Furthermore, microCT analysis of femur from roscovitine treated 13-week old female BALB/c mice revealed significantly higher BV/TV and Cs.Th by elevating osteoblastogenesis *in vivo* (figure 1A). Mechanistically, loss of Cdk5 promotes phosphorylation of Erk, and subsequently increases osteoblast-specific marker gene expression. We further showed that simultaneous knockdown of Cdk5 and Erk abrogates the increase in osteoblastogenesis, suggesting that Cdk5 regulates osteoblast differentiation through modulation of the MAPK pathway (figure 1B-C).



Conclusion(s): Our findings highlights a previously unknown role of Cdk5 in regulating osteoblast differentiation that could serve as a potential target for an osteo-anabolic therapy to treat osteoporosis.

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Plenary Oral Presentations 3: Progress in Translational Research

PLO13

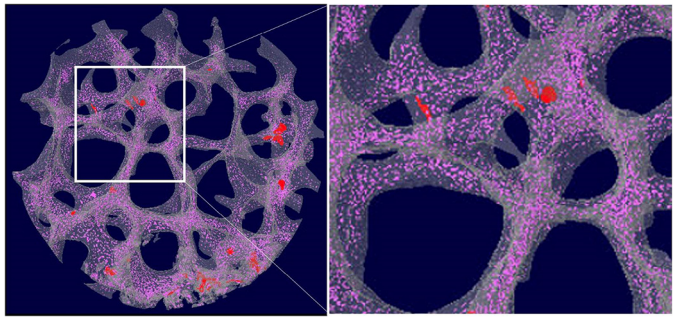
Elevated levels of active Transforming Growth Factor β1 in the subchondral bone relate spatially to cartilage loss in human knee osteoarthritis

Dzenita Muratovic[a], David Findlay[a], Halimatulmasrini Hajimasri[a], Catherine Stapledon[a], Yea Rin Lee[a], Bogdan Solomon[a,b], Xu Cao[c], Gerald Atkins[a], Julia Kuliwaba[a]
[a]The University of Adelaide, Discipline of Orthopaedics and Trauma/ Adelaide Medical School, Adelaide, Australia
[b]The Royal Adelaide Hospital and Central Adelaide Local Health Network, Orthopaedic and Trauma Service, Adelaide, Australia
[c]Johns Hopkins University, Department of Orthopaedic Surgery, Baltimore, United States

Background/Introduction: Over-activity of transforming growth factor beta 1 (TGFβ1) in subchondral bone has a direct causal role in rodent osteoarthritis (OA) which can be attenuated by neutralisation of TGFβ1 activity. In human OA, direct relationship with OA progression has not been demonstrated.

Purpose: The aim of this study was to investigate whether the level of active TGFβ1 protein in the subchondral bone associates with the structural, cellular and molecular parameters that are characteristic of human knee OA.

Methods: With informed consent and institutional approval, tibial plateaus were collected post-operatively from 35 arthroplasty patients (66±9 years old). Bone samples were obtained from regions below macroscopically present cartilage (CA+) and regions below severely degenerated/depleted cartilage (CA-), processed for measurement of active TGFβ1 concentration and gene-specific mRNA expression by RT-PCR, imaged by synchrotron micro-CT to obtain bone quality parameters (Figure 1), and histologically examined to determine OARSI grade, osteocyte, osteoclast and vascular density.



Results: Bone from CA- regions (mean OARSI grade=6) compared to bone from CA+ regions (mean OARSI grade=3) was characterised by higher concentration of active TGFβ1 protein ($p<0.05$), increased RANKL/OPG mRNA ratio ($p<0.05$), more sclerotic bone ($p<0.05$), larger and greater numbers of osteocyte lacunae ($p<0.05$), increased osteocyte ($p<0.0005$) and osteoclast cell density ($p<0.05$) and increased marrow ($p<0.05$) and bone matrix vascular density ($p<0.005$).

Conclusion(s): Increased levels of active TGFβ1 in the subchondral bone associates spatially with disease severity and impaired overall bone quality. Importantly, these findings suggests that in human OA TGFβ1 inhibition could be a potential new therapeutic option to prevent or reduce disease progression.

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PLO14

Deletion of Transferrin Receptor 2 (Tfr2) aggravates inflammation and bone erosion by promoting macrophage activation in inflammatory arthritis

Maria G. Ledesma-Colunga[a], Ulrike Baschant[a], Lorenz C. Hofbauer[a,b], Martina Rauner[a]
[a]Department of Medicine III, Technische Universität Dresden, Dresden, Germany
[b]Center for Healthy Aging, Technische Universität Dresden, Dresden, Germany

Background/Introduction: Rheumatoid arthritis is a progressive articular inflammatory disease with frequent synovial iron

deposition. Transferrin receptor 2 (Tfr2) is a focal point in the regulation of systemic iron levels and bone mass. Loss of Tfr2 function results in hemochromatosis, an iron overload disease.

Purpose: To investigate whether Tfr2 deletion and/or iron overload influences/affects the pathogenesis of inflammatory arthritis.

Methods: We analyzed iron overloaded Tfr2-deficient mice (*Tfr2*$^{-/-}$) and their wild-type (*Tfr2*$^{+/+}$) littermate controls as well as mice lacking Tfr2 only in myeloid cells (*Tfr2*$^{fl/fl}$*-LysMCre+*, normal iron loading) for their capacity to develop K/BxN serum transfer arthritis (STA). Arthritis severity was assessed by clinical scores of paw swelling, and hind paws were collected for micro-CT, histomorphometry, qPCR, and flow cytometry.

Results: *Tfr2*$^{-/-}$ mice developed more pronounced joint swelling, synovial hypertrophy, and bone erosion [2-fold; $p<0.05$] with enhanced mRNA expression of *Il1b*, *Il6*, *Cxcl10,* and *Inos* [2-, 4-, 3.4-, 2-fold; $p<0.05$] as compared to *Tfr2*$^{+/+}$ mice. Furthermore, myeloid cell infiltration including neutrophils (Gr-1^{pos}) and macrophages/monocytes (F4/80^{pos}) at day 7 was increased [2.7-, 1.8-fold; $p<0.05$] in the joints of *Tfr2*$^{-/-}$ mice. To elucidate whether Tfr2 has a direct role in the pathogenesis of arthritis or whether the effects are mediated via the systemic iron overload, we induced STA in *Tfr2*$^{fl/fl}$*-LysMCre+* mice, which showed increased disease development with enhanced synovial hypertrophy and bone erosion [1.4-fold; $p<0.05$] compared to *Cre-* control littermates. Since macrophages regulate iron availability and innate immunity, we hypothesized that Tfr2-deficiency would polarize macrophages toward a pro-inflammatory state (M1-macrophages) that contributes to arthritis progression. Indeed, *Tfr2*$^{-/-}$ macrophages stimulated with IFN-γ showed increased expression of M1 cytokines *Il1b*, *Il6*, *Cxcl10,* and *Inos* [2-, 2-, 1.5-, 1.5-fold; $p<0.05$] and a prolonged STAT1 activation compared to *Tfr2*$^{+/+}$ macrophages.

Conclusion(s): Taken together, these findings suggest a protective role of endogenous Tfr2 on the progression of arthritis.

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PLO15

Single cell mapping of osteoclasts reveals their heterogeneity and highlights the importance of TLR2 in controlling inflammatory osteoclastogenesis

Julia Halper[a,b], Maria-Bernadette Madel[a,b], Maria Firulyova[c], Konstantin Zaitsev[c], Matthieu Rouleau[a], Stoyan Ivanov[d], Abdelilah Wakkach[a], Claudine Blin-Wakkach[a]

[a]*LP2M/UMR7370, Université Côte d'Azur- CNRS, Nice, France*

[b]*Both authors contributed equally to the work, Université Côte d'Azur, Nice, France*

[c]*Computer Technologies Department, ITMO University, Saint Petersburg, Russia C.I.S.*

[d]*C3M, Université Côte d'Azur- INSERM, Nice, France*

Background/Introduction: Osteoclasts (OCLs) have long been regarded as a single population of bone-resorbing cells. However, recent investigations unravelled diverse phenotypic and functional OCL characteristics in healthy and pathological conditions. We previously showed that monocyte-derived OCLs (MN-OCLs) induce immune tolerance and differ from dendritic cell-derived OCLs (DC-OCLs) that stimulate inflammatory responses. *In vivo*, OCLs originate from different progenitors, which can influence their phenotype and function.

Purpose: Our aim was to investigate the heterogeneity of OCLs obtained from total bone marrow (BM) cells.

Methods: We performed single cell-RNAseq on BM-derived OCLs to set in comparison with bulk-RNAseq data from MN-/DC-OCLs.

Results: We identified different clusters, all expressing a classical OCL gene signature (adjusted p-value<0.0001) as well as an individual transcriptomic profile. When compared with MN-OCLs or DC-OCLs gene signatures (168 and 738 genes, respectively, p<0.05, Fold Change>2), distinct clusters were highlighted (Fig1a-b). The cluster highlighted by the DC-OCL signature showed 89 genes significantly overexpressed in comparison to the residual clusters and associated with immune processes, including the DC-OCL markers Cx3cr1 (adj.p-value<0.0001) and TLR2 (adj.p-value<0.0001). This suggests that this cluster may be related to pathological conditions. Accordingly, in regards to osteoporosis, a higher proportion of OCLs expressed Cx3cr1 and TLR2 when derived from ovariectomized mice (p<0.0001 and p<0.05, respectively) compared to Sham controls (Fig1c-d) and TLR2 agonists inhibit osteoclastogenesis from OVX but not Sham mice (p<0.001).

Conclusion(s): Our results underline OCL diversity and ongoing investigations will enable efficient phenotypic and functional distinction between the subsets, to unravel strategies to selectively target those OCLs participating in pathological bone destruction.

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PLO16

De novo serine synthesis regulates chondrocyte proliferation during endochondral ossification

Steve Stegen[a], Ingrid Stockmans[a], Karen Moermans[a], Peter Carmeliet[b], Geert Carmeliet[a]

[a]*KU Leuven, Clinical and Experimental Endocrinology CHROMETA, Leuven, Belgium*

[b]*KU Leuven, Angiogenesis and Vascular Metabolism - VIB, Leuven, Belgium*

Background/Introduction: The avascular nature of cartilage tissue implies that chondrocytes exhibit specific ways of nutrient acquisition and metabolite synthesis to fulfill their anabolic functions. Serine is a critical amino acid for protein synthesis, but also nucleotide generation.

Purpose: Our unbiased metabolomics analysis showed that chondrocytes synthesize roughly 50% of the intracellular serine pool via glucose even when sufficient extracellular serine is present, but the functional benefit of this metabolic plasticity is unknown.

Methods: To investigate the role of the serine synthesis pathway (SSP) in chondrocytes, we deleted the rate-limiting enzyme phosphoglycerate dehydrogenase (PHGDH) using Col2a1-Cre mice.

Results: PHGDH knockout mice displayed progressively stunted growth (tibia length: -15%, p<0.01), which was explained by a reduction in the proliferating zone of the growth plate (-10%, p<0.01). Mechanistically, we found that the SSP is essential for purine and pyrimidine generation to sustain chondrocyte proliferation, without affecting other cellular processes like survival and matrix synthesis. Accordingly, nucleotide supplementation completely rescued the proliferation defect in PHGDH-deficient cells in vitro. The functional benefit of de novo serine synthesis was further underscored in conditions of dietary serine withdrawal, as serine-starved chondrocytes fully compensate by stimulating PHGDH-mediated serine synthesis in

vitro and in vivo (+300%, p<0.01). Mechanistically, serine deprivation increases ATF4 signaling (+550%, p<0.01), which transactivates SSP-related enzymes to avoid intracellular serine depletion and chondrocyte dysfunction.

Notably, the importance of the SSP in chondrocytes was not only evident during bone development, but also during fracture healing as pharmacological inhibition of PHGDH blocked the formation of a cartilaginous callus (-30%, p<0.05). In contrast, feeding mice a serine-free diet did not affect bone repair due to a compensatory upregulation of the SSP.

Conclusion(s): Collectively, our data indicate that the SSP safeguards chondrocyte anabolism upon fluctuations in serine availability. This metabolic flexibility is especially relevant during fracture healing, when the damaged surrounding vasculature hinders proper nutrient supply.

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PLO17

Kinetic reconstruction of the cancellous (Cn) and endocortical (Ec) remodelling unit reveals a net positive bone balance (BB) after 12 months of treatment with romosozumab

Erik F. Eriksen[a], Rogely W. Boyce[b], Yifei Shi[c], Jacques P. Brown[d], Stéphane Horlait[e], Cesar Libanati[f], Roland Chapurlat[g], Pascale Chavassieux[g]

[a]*Oslo University Hospital, Department of Endocrinology- Morbid Obesity and Preventive Medicine, Oslo, Norway*

[b]*Amgen Inc., Department of Comparative Biology and Safety Sciences, Thousand Oaks- CA, United States*

[c]*Amgen Inc., Global Biostatistical Science, Thousand Oaks- CA, United States*

[d]*CHU de Quebec Research Centre and Laval University, Department of Medicine, Quebec, Canada*

[e]*Amgen Inc., Bone Medical Strategy, Boulogne Billancourt, France*

[f]*UCB Pharma, Bone Medical Strategy, Brussels, Belgium*

[g]*INSERM UMR 1033- Université de Lyon, Bone and Chronic Diseases, Lyon, France*

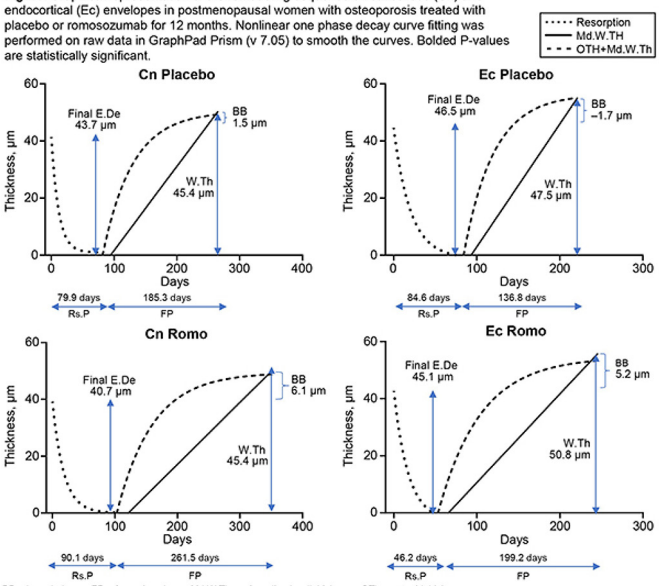
Background/Introduction: Romosozumab-aqqg, a sclerostin inhibitor, leads to a rapid transient increase in modelling-based bone formation and a sustained decrease in resorption.[1,2]

Purpose: Assess the effect of romosozumab on bone formation/resorption in the basic multicellular unit (BMU) and determine integral effects on BMU BB after 12 months (12M) of romosozumab/placebo treatment by performing 3D reconstruction of Cn and Ec BMU[3] on transiliac biopsies from the FRAME trial (NCT01575834).

Methods: Erosion depth (E.De), osteoid thickness (O.Th), and complete/incomplete wall thickness (W.Th) were based on counting lamellae in polarised light. To assess effects on the formative site at 12M, O.Th/mineralised W.Th at osteoid surfaces were classified using a 4-sector system; the sector containing nearly completed formative sites provided the reconstructed W.Th.

Results: On Cn envelopes, romosozumab resulted in a net positive BB vs placebo primarily due to reduction in resorptive cell activity throughout 12M, resulting in a significant reduction in final E.De. Romosozumab also resulted in a net positive BB on Ec envelopes, due to positive effects on the formative site. Consequently, romosozumab significantly increased W.Th in bone packets by 12M. This was not sustained in actively forming packets at the end of treatment, suggesting positive bone-forming effects at the BMU occurred earlier in treatment.

Figure. Graphical representation of the remodeling sequence on cancellous (Cn) and endocortical (Ec) envelopes in postmenopausal women with osteoporosis treated with placebo or romosozumab for 12 months. Nonlinear one phase decay curve fitting was performed on raw data in GraphPad Prism (v 7.05) to smooth the curves. Bolded P-values are statistically significant.



BB = bone balance; FP = formative phase; Md.W.Th = mineralized wall thickness; OTh = osteoid thickness; Final E.De = pre-osteoblast (final) erosion depth; Rs.P = resorptive phase; W.Th = wall thickness

	Placebo N = 31	Romosozumab N = 39	P-value
Remodeling Parameter, µm, median (Q1, Q3)			
Cancellous envelope			
Osteoclast erosion depth	18.1 (14.5, 22.4)	15.4 (11.1, 18.4)	**0.027**
Mononuclear cell erosion depth	33.6 (27.7, 38.9)	29.3 (22.4, 34.1)	0.067
Final erosion depth	43.7 (40.0, 49.0)	40.7 (36.7, 46.2)	**0.05**
Wall thickness	45.4 (41.5, 48.8)	45.4 (41.6, 50.6)	0.57
Reconstructed wall thickness	51.2 (46.2, 55.5)	51.2 (46.7, 57.2)	0.64
Bone balance	1.5 (−6.1, 6.1)	6.1 (1.5, 9.0)	**0.012**
Endocortical envelope			
Osteoclast erosion depth	19.2 (14.9, 23.0)	12.8 (9.6, 17.1)	**<0.001**
Mononuclear cell erosion depth	33.1 (28.6, 40.9)	26.7 (21.5, 30.7)	**<0.001**
Final erosion depth	46.5 (43.3, 55.8)	45.1 (39.2, 52.9)	0.33
Wall thickness	47.5 (41.9, 52.1)	50.8 (45.4, 53.3)	**0.037**
Reconstructed wall thickness	56.0 (48.9, 63.1)	56.5 (49.9, 62.1)	0.84
Bone balance	−1.7 (−6.6, 3.4)	5.2 (−2.0, 11.9)	**0.02**

Conclusion(s): Net decreases in resorptive cell activity in Cn bone and net increases in osteoblastic function in Ec bone likely contribute to progressive increases in bone mass and microarchitectural improvements with romosozumab across 12M of treatment.

References: 1. Chavassieux P. *JBMR* 2019;34:1597-1608. 2. Eriksen EF. *ASBMR* 2019; 3. Eriksen EF. *Endocr Rev* 1986;7:379-408.

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PLO18

Investigating Denosumab discontinuation in the TgRANKL osteoporotic mouse model

Vagelis Rinotas[a,b], Eleftheria-Dimitra Ntouskou[a], Melina Dragolia[a], Vasileios Ntafis[a], Maria Yavropoulou[c], Athanasios Anastasilakis[d], Polyzois Makras[e], Eleni Douni[a,b]

[a]*Biomedical Sciences Research Center "Alexander Fleming", Institute for Bioinnovation, Athens, Greece*

[b]*Agricultural University of Athens, Department of Biotechnology, Athens, Greece*

[c]*Medical School- National and Kapodistrian University of Athens, Endocrinology Unit- 1st Department of Propaedeutic and Internal Medicine, Athens, Greece*

[d]*424 General Military Hospital, Department of Endocrinology, Thessaloniki, Greece*

[e]*251 Hellenic Air Force & VA General Hospital, Department of Endocrinology and Diabetes and Department of Medical Research, Athens, Greece*

Background/Introduction: Even though the RANKL inhibitor Denosumab (Dmab) offers an effective and safe therapy for

postmenopausal osteoporosis, its discontinuation is associated with a rebound phenomenon resulting in rapid bone loss and an increased risk of multiple vertebral fractures. Thus, effective subsequent treatments are urgently needed for the management of Dmab discontinuation. Dmab is ineffective in osteoporotic mouse models since it cannot bind to mouse RANKL.

Purpose: In the current study we modelled Dmab discontinuation in our TgRANKL osteoporotic transgenic mice that overexpress human RANKL.

Methods: The experimental groups included: i)wild-type (WT) mice untreated, ii)TgRANKL untreated, iii)TgRANKL treated with Dmab for 18 weeks, iv)TgRANKL treated with Dmab for 6 weeks and then untreated for 12 weeks (discontinuation group), v)TgRANKL treated with Dmab for 6 weeks and then changed to zoledronate for 12 weeks. To monitor bone loss progression *in vivo*, we quantified bone density with an x-ray scanner, while femurs were assessed with histological analysis and micro-CT.

Results: Our results showed that TgRANKL femurs displayed significant trabecular bone loss compared to WT (BV/TV %, WT:10.00 $\pm$5.78, TgRANKL:1.09$\pm$1.13, $p<0.001$), while continuous Dmab treatment of TgRANKL mice completely inhibited bone resorption and increased trabecular bone volume above the values of WT mice (BV/TV %, 22.63$\pm$6.88, $p<0.001$). Notably, in the discontinuation group we detected a rapid severe bone loss as estimated by the significantly reduced trabecular bone volume, which reached similar levels (BV/TV %, 1.41$\pm$1.32, $p>0.05$) with the untreated osteoporotic TgRANKL mice. On the contrary, TgRANKL mice that were initially treated with Dmab and then changed to zoledronate maintained the bone mass gained by Dmab (BV/TV %, 24.72$\pm$2.96, $p>0.05$). Gene expression analysis confirmed the various treatment effects on the skeletal system.

Conclusion(s): In conclusion, we established a Dmab discontinuation mouse model to investigate the underlying molecular mechanisms and evaluate various therapeutic approaches following Dmab discontinuation at the preclinical level.

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Concurrent Oral Presentations

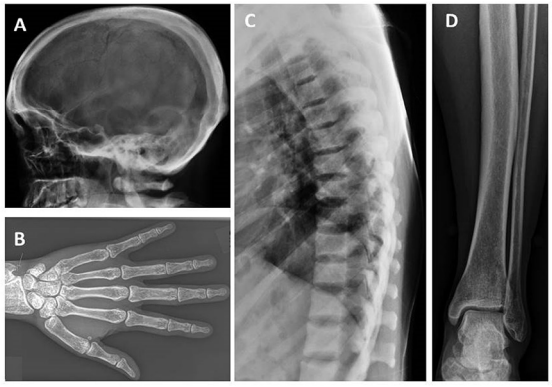
Concurrent Oral Presentations 1: Clinical / Public Health: Diseases other than Osteoporosis

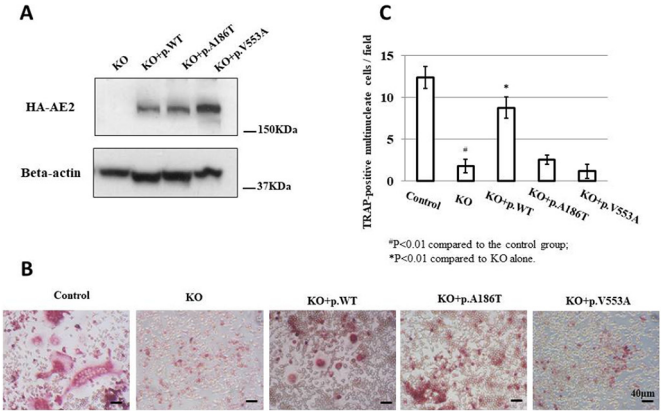
COP01

Identification of SLC4A2 as a new causal gene for osteopetrosis in human

Jingyi Xue[a,b], Zheng Wang[a], Long Guo[a], Shiro Ikegawa[a]
[a]RIKEN, Laboratory for Bone and Joint Diseases, Tokyo, Japan
[b]Yokohama city university, Human Genetics, Yokohama, Japan

Background/Introduction: Osteopetrosis is a group of rare genetic disorders characterized by increased bone density.





Osteopetrosis is genetically heterogeneous and nine causal genes have been reported. Nevertheless, no mutation is found among these reported genes on some patients, suggesting unknown causal genes exist. Anion exchanger 2 (AE2, encoded by *SLC4A2*) exports carbonate ions and imports chloride ions to maintain the intracellular pH. AE2 is highly expressed in osteoclasts. *SLC4A2*-deficient mice and cattle display osteopetrosis associated with dysfunctional osteoclasts; however, no mutations in human *SLC4A2* have been reported to date in the context of osteopetrosis.

Purpose: To identify *SLC4A2*-associated osteopetrosis in human.

Methods: Whole exome sequencing and Sanger sequencing were used to detect the variant in the patient. Pathogenicity of the variants was assessed by a gene knockout-rescue system using mouse macrophage cell line, RAW 264.7.

Results: By whole exome sequencing, we identified novel compound heterozygous variants in *SLC4A2* (NM_003040.4: c.556G>A [p.A186T] and c.1658T>C [p.V553A]) in an osteopetrosis patient. Osteoclast differentiation induced by RANKL showed that osteoclastogenesis was impaired in *Slc4a2*-knockout RAW 264.7 cells. The impairment was rescued by wild-type SLC4A2, but not by mutant SLC4A2.

Conclusion(s): We discovered the first case of *SLC4A2*-associated osteopetrosis patient, which highlights the importance of AE2 in osteoclastogenesis in human.

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COP02

Femoral anteversion (FNA) in individuals with X-linked hypophosphatemia (XLH)

Matteo Scorcelletti[a], Serhan Kara[b], Lothar Seefried[c], Jochen Zange[b], Jörn Rittweger[b], Alex Ireland[a]
[a]Manchester Metropolitan University, Science and Engineering, Manchester, United Kingdom
[b]Institute of Aerospace Medicine DLR, Division of Muscle and Bone Metabolism, Cologne, Germany
[c]University of Würzburg, Orthopedic Department, Würzburg, Germany

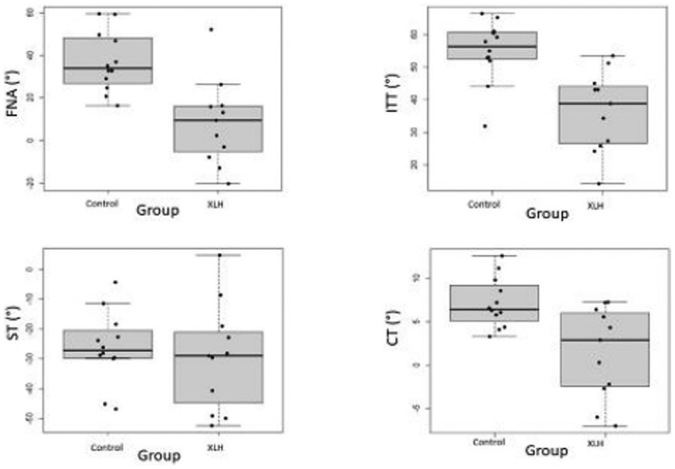
Background/Introduction: XLH is a rare genetic condition which affects phosphate metabolism, resulting in osteomalacia. Individuals with XLH are also at risk of lower limb deformities and early onset of hip osteoarthritis. These two factors may be linked, as abnormal FNA (femoral torsion) is a risk factor for hip osteoarthritis. The contributions of regional femoral torsion *e.g.* intertrochanteric torsion (ITT), shaft torsion (ST) and condylar torsion(CT) to FNA

differ between clinical groups and are important when planning femoral osteotomies to correct FNA.

Purpose: This study aimed to compare FNA and regional femoral torsion of the femur between adults with XLH and controls.

Methods: 13 individuals with XLH (5 male, age 49±9y) and 12 age, sex and weight-matched control participants (7 male, age 49±8y) were recruited following ethical approval and informed consent. Magnetic resonance imaging (MRI) scans of the femur were obtained, from which FNA, ITT, ST and CT were measured. Data were normally distributed, therefore group differences were assessed using t-tests.

Results: FNA was 29° lower in individuals with XLH than controls ($p<0.005$). This resulted mainly from lower ITT ($p<0.001$) and in part CT ($p<0.05$) whereas ST was similar in the two groups (Fig. 1).



Conclusion(s): We observed differences in FNA and region-specific femoral torsion in individuals with XLH compared to controls. These differences may contribute to clinical problems such as hip osteoarthritis common in XLH. Information on region-specific differences may be useful in planning corrective surgeries. Future work should examine how pharmacological and other interventions in this group affect FNA.

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COP03

Pregnancy-associated fracture risk in women with osteogenesis imperfecta, a nationwide register-based SCCS

Emilie Karense Lykking[a], Heidi Kammerlander[b], Fleur Van Dijk[c], Daniel Prieto-Alhambra[d], Bo Abrahamsen[e], Lars Folkestad[f]

[a]*Odense University Hospital / University of Southern Denmark, Department of Endocrinology / Institute of Health Sciences, Odense, Denmark*

[b]*Hospital Lillebaelt, Department of Obstetrics and gynaecology, Kolding, Denmark*

[c]*London North West Healthcare NHS Trust, North West Thames Regional Genetics Service, London, United Kingdom*

[d]*University of Oxford, Csm-NDORMS, Oxford, United Kingdom*

[e]*Holbaek Hospital / University of Southern Denmark, Department of Medicine / OPEN, Holbaek, Denmark*

[f]*Odense University Hospital / University of Southern Denmark, Department of Endocrinology / Department of Clinical Research, Odense C, Denmark*

Background/Introduction: Osteogenesis imperfecta (OI) is a hereditary disorder of the connective tissue with a heterogeneous clinical presentation. A hallmark of OI is frequent fractures occurring with little or no trauma. Pregnancy and lactation are periods of increased fetal demand for calcium known to often result in an asymptomatic and fully reversible decrease in maternal Bone Mineral Density. Fracture risk associated with this bone loss among women with OI has not yet been evaluated.

Purpose: To evaluate the fracture rates and risk in the short and longer term associated with pregnancy.

Methods: Self-controlled case series 12- and 19- months prior to conception compared to a period of 12- and 19 months, respectively, postpartum among women with OI.The study is based on register data from the Danish National Patient Register. All women registered in the Danish National Patient Register with a WHO International Classification of Diseases 8th or 10th edition code for OI who gave birth one or more times in the period between 01.01.1995-31.12.2018 and who had a 12 or 19 months pre- and postpartum observation period were included.

Results: We found an incidence rate (IR) 12 months prior to conception of 59.9 [95%CI 22.8-97] per 1000 person years and an IR 12 months postpartum of 29.9 [95%CI 3.7-56.18]. Comparing pre- and post-pregnancy periods we found an incidence rate ratio (IRR) of 0.5 [95%CI 0.17-1.46]. Adjusting for parity and age at delivery did not significantly change in IRR. For the 19 months window the IR per 1000 person years pre-pregnancy was 58.28 [95%CI 36.16-87.77] and the IR postpartum 51.12 [95%CI 23.33-78.91], leading to an IRR of 0.87 [95%CI 0.40-1.82].

Conclusion(s): We found no evidence that the anticipated physiological decline in BMD during pregnancy and lactation leads to a higher risk of fractures in women with OI.

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COP04

Breast calcification chemistry as a biomarker for progression of in-situ breast cancer

Sarah Gosling[a], Doriana Calabrese[b], Emily Arnold[a], Jayakrupakar Nallala[b], Charlene Greenwood[c], Nicholas Stone[b], Keith Rogers[a]

[a]*Cranfield University, Cranfield Forensic Institute, Swindon, United Kingdom*

[b]*University of Exeter, School of Physics and Astronomy, Exeter, United Kingdom*

[c]*Keele University, School of Chemical and Physical Sciences, Keele, United Kingdom*

Background/Introduction: Breast microcalcifications are deposits of calcium oxalate, found mostly in benign tissue, or calcium phosphate in the form of hydroxyapatite, found in benign and malignant tissue. Differences in the crystallographic properties and chemical make-up of hydroxyapatite breast microcalcifications have previously been noted in differing breast pathologies.

Purpose: Ductal carcinoma in-situ (DCIS) is a precancerous breast lesion, which has the potential to form invasive breast cancer. Currently there are no definitive markers to determine DCIS invasiveness, therefore this work aims to elucidate differences in the calcification chemistry between invasive and non-invasive cases of DCIS, ultimately developing a novel biomarker for DCIS progression.

Methods: 75 formalin fixed paraffin embedded archive breast tissue samples were used subject to NHS REC approval (ref. 18/LO/0945). X-ray diffraction was carried out at 12keV on beamline i18 at Diamond Light Source, UK to determine crystallographic properties

of 279 breast calcifications. SEM-EDS experiments were carried out on a Hitachi SU3500 at 11kV under low vacuum.

Results: Significant differences ($P < 0.05$) were observed in the proportion of magnesium whitlockite found as a secondary phase in breast calcifications from invasive (3.51 %) and non-invasive (2.82 %) DCIS samples. Additionally, crystallographic features of hydroxyapatite, the bulk of calcifications in both cases, were found to differ between the two groups. The d-spacing between crystallographic planes, was significantly ($P < 0.001$) larger in invasive compared to non-invasive DCIS cases. Finally, the calcium to phosphate ratio measured using EDS was significantly lower ($P < 0.001$) in invasive samples (1.60) compared to non-invasive samples (1.67), which more closely reflected stoichiometric hydroxyapatite.

Conclusion(s): Differences in calcification chemistry and crystallographic structure between invasive and non-invasive DCIS cases have been demonstrated in this study. Therefore, calcification chemistry is a key candidate for novel DCIS progression biomarkers and could ultimately lower treatment expenditure and improve patient quality of life by reducing overtreatment.

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COP05

Dxa-based bone strain index: A new tool to evaluate bone quality in primary hyperparathyroidism

Gaia Tabacco[a], Anda Mihaela Naciu[a], Carmelo Messina[b], Luca Rinaudo[c], Roberto Cesareo[d], Stefania Falcone[e], Silvia Manfrini[a], Nicola Napoli[a], John Paul Bilezikian[f], Fabio Massimo Ulivieri[g], Andrea Palermo[a]

[a]*Campus Bio-Medico University of Rome, Unit of Endocrinology and Diabetes, Rome, Italy*
[b]*IRCCS Istituto Ortopedico Galeazzi, Radiology, Milan, Italy*
[c]*TECHNOLOGIC, Srl, Turin, Italy*
[d]*Santa Maria Goretti Hospital, UOS Malattie Metaboliche, Latina, Italy*
[e]*CTO A. Alesini Hospital, Unit of Endocrinology and Metabolic Diseases, Rome, Italy*
[f]*College of Physicians & Surgeons- Columbia University, Department of Medicine- Division of Endocrinology, New York, United States*
[g]*Casa di Cura, La Madonnina, Milan, Italy*

Background/Introduction: Primary hyperparathyroidism (PHPT) is associated with impaired bone quality and increased fracture risk. Reliable tools for the evaluation of bone quality parameters are not yet clinically available. Bone Strain Index (BSI) is a new metric for bone strength based on Finite Element Analysis from lumbar spine and femoral neck dual X-ray absorptiometry images.

Purpose: To assess the lumbar spine (LS), femoral neck (FN), and total hip (TH) BSI in PHPT compared to controls.

Methods:

Design: Cross-sectional study.

Setting: Outpatient clinic.

Patients: 44 PHPT and 39 age- and sex-matched control subjects.

Main Outcome Measures: LS-BSI, FN-BSI, TH-BSI.

Results: TH bone mineral density (BMD) and 1/3 distal radius BMD were lower in the PHPT group than in controls (TH 0.802±0.13 vs 0.872±0.09, P<0.05; radius 0.565±0.07 vs 0.620±0.06, P<0.001). There were no differences between groups in trabecular bone score (TBS) and T-score adjusted for TBS. BSI was significantly higher at LS (2.20±0.58 vs 1.94±0.48, p=0.003), FN (1.66±0.39 vs 1.40±0.36, p=0.003) and TH (1.46±0.3 vs 1.24 ±0.25, p=0.001) in PHPT. LS-BSI showed moderate accuracy for detecting vertebral fractures [(area under the ROC curve 0.68 (CI:0.52-0.848)]. The best cut-off was set at 2.12 (sensibility 72%, specificity 64%, accuracy 67.4%).

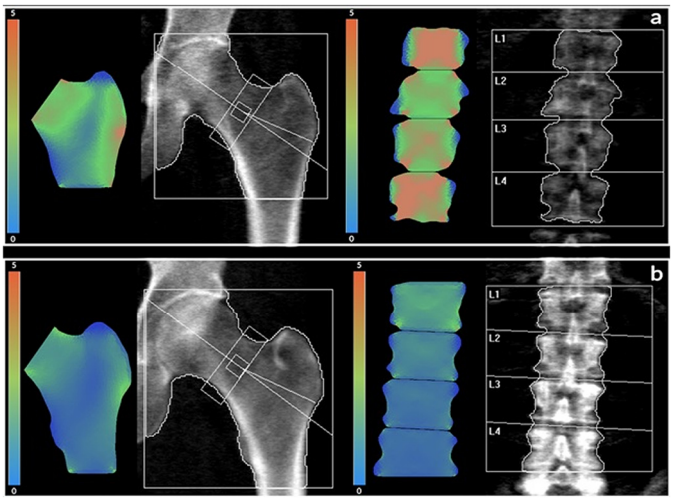

Figure 1. Femoral and Lumbar Spine BSI images in a subject with hyperparathyroidism (a) and in a control subject (b). The corresponding values of BSI were:
a. Femoral neck BSI 2.33; Total Hip BSI 1.94; Lumbar spine BSI 3.02
b. Femoral neck BSI 1.13; Total Hip BSI 1.17; Lumbar spine BSI 0.93.

Conclusion(s): BSI, a DXA-derived bone quality index, is impaired in PHPT and may help to identify PHPT subjects at high risk of fractures.

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COP06

Prevalence of monogenic bone disorders in a Dutch cohort of AFF patients

Wei Zhou[a], Jeroen van Rooij[a], Denise van de Laarschot[a], Zografia Zervou[a], Natasha Appelman-Dijkstra[b], Serwet Demirdas[c], Sarina Kant[c], Annemieke Verkerk[a], M. Carola Zillikens[a]

[a]*Erasmus Medical Center, Department of Internal Medicine, Rotterdam, Netherlands*
[b]*Leiden University Medical Center, Department of Internal Medicine, Leiden, Netherlands*
[c]*Erasmus Medical Center, Department of Clinical Genetics, Rotterdam, Netherlands*

Background/Introduction: Atypical femur fractures (AFFs) have been reported in patients with monogenic bone disorders, such as osteogenesis imperfecta and hypophosphatasia, with or without bisphosphonates use. It is still unknown how AFFs are associated with these monogenic bone disorders.

Purpose: Our aim of this study was to determine the number of AFF patients diagnosed with monogenic bone disorders in a Dutch AFF patient cohort and also how many AFF patients without a clinical suspicion of monogenic bone disorder are carriers of rare variants in genes associated with monogenic bone disorders.

Methods: Sixty AFF patients were seen at two bone expertise centers in Dutch University Hospitals in Rotterdam and Leiden. According to the clinical files, the AFF patients were classified into three groups: 1) genetically confirmed with a pathogenic variant in a known gene causing monogenic bone disorder; 2) clinically suspected monogenic bone disorders without known pathogenic variants; 3) AFF patients without suspicion for monogenic bone disorders. We performed whole exome sequencing (WES) and classified the identified rare variants based on the American College of Medical Genetics and Genomics (ACMG) classification guideline.

Results: Seven (12%) AFF patients had a clinically suspected and genetically confirmed monogenic bone disorders (first group). Additionally, we identified three patients clinically suspected of a monogenic bone disorder harbouring a heterozygous likely pathogenic variant: c.G964A in *COL1A2* (in two sisters) and c.2827 +1G>A in *LRP5*. An AFF patient without suspicion of monogenic bone disorders, but with high bone mineral density at the spine (T-score=3.3) and low-normal bone mineral density at the hip (T-score=-1.2), was found to carry a heterozygous likely pathogenic variant c.C2008T in *TCIRG1*.

Conclusion(s): Approximately15% of AFF patients presenting to bone expertise centers do have an underlying monogenic bone disease, further strengthening the hypothesis of a genetic cause of AFF. More genetic studies are needed to further determine the genetic architecture of AFF.

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Concurrent Oral Presentations 1: Basic / Translational: Cancer, Regeneration and Stem Cells

COP07

Extracellular vesicles from metastatic prostate cancer modulate osteoblastic functions

Giulia Furesi, Martina Rauner, Lorenz C. Hofbauer
Technische Universität Dresden, Department of Medicine III and Center for Healthy Aging, Dresden, Germany

Background/Introduction: Bone metastases are a hallmark of advanced prostate cancer (PCa). Interaction between tumor and the skeleton is crucial for cancer organotropism. Extracellular vesicles (EVs) mediate long-distance communication between cells by delivering bioactive-molecules. However, how tumor-derived EVs promote a favorable bone-niche for PCa metastasis remains unclear.

Purpose: The present study aimed to evaluate the effects of PCa-EVs on osteoblasts (OB) *in vitro and in vivo*.

Methods: EVs were isolated from the murine PCa cell line RM1-BM. Internalization of EVs by primary OB was detected using flow cytometry. Additionally, OB were treated with PCa-EVs to assess the effects on OB viability and mineralization capacity. Also, RNAseq was performed to investigate the gene expression of OB after EV treatment and identify exosomal microRNAs that potentially regulate OB functions. simiRNA-mediated knockdown was used to examine the effect of EVs-related microRNAs on OB. Finally, the impact of PCa-EVs on osteogenesis *in vivo* was evaluated using a calvarial defect mouse model.

Results: Flow cytometry revealed the uptake of PCa-EVs into OB as early as after 1h. OB treated with PCa-EVs showed a dose-dependent increase of cell viability [+65%, $P<0.001$] compared to controls, while mineral deposition was significantly reduced [-37%, $P<0.001$]. Gene-set enrichment analysis showed downregulation of osteogenic markers [$P<0.02$] and upregulation of the inflammatory factors [$P<0.001$] in OB treated with PCa-EVs. Exosomal transcriptomic profiling revealed high abundance of 3 microRNAs (miR30e-5p, miR26a-5p, miR27a-3p) that have been linked with OB activity. μCT analysis of calvarias revealed significantly decreased bone defect healing after treatment with PCa-EVs ($P< 0.01$), an effect that was reversed by silencing either one of the three EVs-related microRNAs in PCa cells ($P<0.01$).

Conclusion(s): Our results highlight the importance of EVs in the intercellular crosstalk between PCa cells and OB *in vitro* and *in vivo*. Moreover, our data suggest that miRNA may be crucial mediators of this interaction.

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COP08

Transcriptome analysis of Notch2 High Breast Cancer (BrCa) cells revealed new molecular determinants of BrCa cellular dormancy in the bone/bone marrow microenvironment

Michela Ciocca, Antonio Maurizi, Iona Norwood, Nadia Rucci, Anna Teti
University of L'Aquila, Department of Biotechnological and Applied Clinical Sciences, L'Aquila, Italy

Background/Introduction: BrCa cells that interact with Spindle-shaped N-cadherin^{+} Osteoblasts (SNOs) are recognised to become dormant through a Notch2-dependent mechanism and to keep a stem-phenotype.

Purpose: This work was aimed at identifying new molecular determinants of BrCa cellular dormancy in the bone/bone marrow microenvironment.

Methods: We performed an unbiased RNA dSeq analysis of Notch2^{High} and Notch2^{Low} MDA-MB231 (MDA) cells.

Results: Bioinformatics and biostatistics revealed an enrichment of pathways involved in *Neuroactive ligand-receptor interaction*, *Leukocyte trans endothelial migration* and *cancer* in the Notch2^{High} MDA cells ($p<0.01$). On the contrary, in line with the dormant phenotype, *metabolic pathways* were significantly unenriched in the Notch2^{High} vs Notch2^{Low} MDA ($p=0.05$). Interestingly, Gene Set Enrichment analysis showed an enrichment in the pluripotency signature of Notch2^{High} compared to Notch2^{Low} MDA (NES=1.5; $q=0.01$), consistent with the stem phenotype we previously discovered in these cells. Data derived from the RNA dSeq were then used to extrapolate new tumor cell dormancy determinants according to their cell surface localization, stem potency and drug targetability. We identified 10 mRNAs differentially expressed in Notch2^{High} vs Nocth2^{Low} MDA cells. Real time RT-PCR, immunofluorescence and Western blot analyses confirmed a higher expression of the *CD177*, *CD163L1*, *CD55* and *CD2AP* genes in Notch2^{High} vs Notch2^{Low} MDA cells (+2.4;+2.5;+1.6;+1.2 fold, respectively, $p<0.05$). In silico analysis revealed the presence of interactants for the markers identified in the dormant BrCa cells in the SNOs counterpart. Consistently, real time RT-PCR analysis confirmed higher expression of the *CD177* interactants, *Itgam*, *Plaur* and *Ceacam1* (+18.8;+7.6;+5 fold, respectively, $p<0.05$) and of the *CD163L1* interactant, *Cd180* (+23.4 fold; $p=0.04$) in the SNOs compared to the NON-SNOs. Expression of *CD55* and *CD2AP* interactants was unremarkable.

Conclusion(s): Our data demonstrated the presence of new potentially targetable molecular patterns that could mediate the BrCa-SNO interaction, possibly regulating the tumor cellular dormancy in the bone/bone marrow microenvironment.

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COP09

Fibronectin derived from osteoblastic progenitors suppresses tumour growth

Alexander Lubosch, Franziska Wirth, Caren Zöller, Inaam Nakchbandi
University of Heidelberg, Institute of Immunology, Heidelberg, Germany

Background/Introduction: Mesenchymal stromal cells in the bone marrow modulate the immune response against cancer. While some studies indicate a tumor-promoting effect of stromal cells, there is also evidence that they act as tumor suppressors.

Purpose: Our group already showed that fibronectin from differentiated osteoblasts promotes tumor growth indirectly by modulating the immune response. We next aimed to determine whether pre-osteoblastic fibronectin might also modify tumor growth.

Methods: Stromal cells, containing vascular and osteoblastic progenitors, were isolated from murine bone marrow and subcutaneously injected together with 500,000 B16 melanoma cells (at a ratio of 0.1:1 (MSC:B16)).

Results: Wildtype stromal cells reduced tumor growth by 55% ($p < 0.001$). Next, fibronectin was deleted in most of the cells in the bone marrow using the Mx promoter attached to Cre recombinase in animals homozygous for floxed fibronectin genes (Mx-conditional knockout: Mx-FN-cKO) after injection of poly(I:C). This led to an increase of tumor size by 230% compared to wildtype stromal cells, and suggests that stromal-derived fibronectin is required for tumor suppression. We next used stromal cells from different cKO models. The knockout of fibronectin in differentiating osteoblasts using the collagen a1(I) promoter (Col-FN-cKO) and in vascular progenitors using the Vav promoter (Vav-FN-cKO) did not change tumor growth compared to wildtype stromal cells ($p = n.s.$). Interestingly, the knockout of fibronectin in pre-osteoblasts using the Osterix promoter (Osx-FN-cKO) showed no suppression of tumor growth and resulted in 270% bigger tumors compared to wildtype cells ($p<0.05$). In contrast, by deleting b1 integrin in pre-osteoblasts (Osx-b1-cKO), suppression of tumor growth was similar to wildtype stromal cells.

Conclusion(s): Taken together, these data suggest that pre-osteoblasts inhibit cancer growth. This effect requires the production of fibronectin by a subpopulation that uses the osterix promoter, but does not itself respond to fibronectin.

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COP10

The Rho GTPase Cdc42 modulates osteoblast number and function depending on the time of its expression

Franziska Wirth[a], Alexander Lubosch[a], Caren Zöller[a], Katrin Huck[b], Inaam Nakchbandi[a]

[a]*University Hospital Heidelberg, Institute for Immunology, Heidelberg, Germany*

[b]*DKFZ Heidelberg, CCU Neuroimmunology and Brain Tumor Immunology, Heidelberg, Germany*

Background/Introduction: Osteoblasts support bone formation and also affect hematopoiesis and modulate immune cell behavior. Such complex effects require incorporating signals from several pathways. Rho GTPases represent molecules present at the crossroads of several signaling pathways.

Purpose: We therefore aimed to characterize the influence of Cdc42, a Rho GTPase in osteoblasts.

Methods: Cdc42 was deleted in early osteoblasts using the Osterix (Osx) promoter to drive Cre expression in Cdc42 floxed homozygous mice (Osx-conditional knockout: Osx-cKO) or using the collagen a1(I) promoter active in differentiating osteoblasts (Col-cKO).

Results: Bone mineral density was diminished (CT 290 vs. Osx-cKO 246, $p<0.0001$; CT 262 vs. Col-cKO 243 mg/cm^3, $p<0.05$). Histomorphometry showed decreased osteoblast function in Osx-cKO (Adjusted apposition rate: CT 3 vs. Osx-cKO 1.7 um/d, $p<0.005$), and osteoblast number in Col-cKO (CT 42 vs. Col-cKO 28 ObN/mm, $p<0.05$). This confirms the importance of Cdc42 in osteoblasts.

Evaluation of hematopoiesis revealed that deletion of Cdc42 in early osteoblasts (Osx-cKO) increased common myeloid progenitors (CMPs) in the bone marrow (CT 0.29 vs. Osx-cKO 0.34%, $p<0.05$) as well as erythrocytes and platelets in peripheral blood which are generated from the progeny of the CMPs called the megakaryocyte erythroid progenitors (Platelets in CT 1352 vs. Osx-cKO 1699 x10^3/ul, $p<0.05$). These changes were not detected when Cdc42 was deleted in differentiating osteoblasts. Using sorted hematopoietic stem and progenitor cells we confirmed an increase in CMPs when Cdc42 is inhibited (CT 4.7 vs. inhibitor 7.5%, $p<0.05$). Further work established that interleukin-4 produced by early osteoblasts, whenever Cdc42 function is diminished, was responsible for increased myelopoiesis (Cdc42-inhibitor 4.2 vs. Cdc42-inhibitor+IL-4-inhibitor 3.1%, $p<0.05$, not significant to untreated control).

Conclusion(s): Thus, Cdc42 is required for healthy bone by regulating early osteoblast function and the number of differentiating osteoblasts. In addition, its expression in early osteoblasts modulates myelopoiesis. This highlights the role of osteoblasts in regulating hematopoiesis.

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COP11

Tensin-3 oppositely regulates bone mesenchymal stromal cell-derived osteogenesis and adipogenesis

Shuang Zhang, Marijke Koedam, Jeroen van de Peppel, Johannes P.T. M. van Leeuwen, Bram van der Eerden

Erasmus MC, Internal medicine, Rotterdam, Netherlands

Background/Introduction: The tightly controlled balance between osteogenic and adipogenic differentiation of bone marrow mesenchymal stromal cells (BMSCs) is critical to maintain bone homeostasis. Age-related osteoporosis is characterized by low bone mass with excessive infiltration of adipose tissue in the bone marrow compartment. The preferential shift of BMSCs differentiation from osteoblast to adipocyte results in bone loss and adiposity. Recently we found Tensin-3 (TNS3) to be an interesting regulator of BMSC differentiation based on microarray expression data in osteoblasts and adipocytes.

Purpose: Identify role of Tensin-3 (TNS3) in BMSCs differentiation.

Methods: TNS3 gene expression during osteogenic and adipogenic differentiation of BMSCs was evaluated by qPCR. Lentiviral-mediated knockdown or overexpression of TNS3 was used to assess its consequences for BMSCs differentiation. The organization of cytoskeleton was examined by immunofluorescent staining. ALP activity, calcium assay, alizarin red staining and Oil red O staining were performed *in vitro* during osteoblast or adipocyte differentiation.

Results: TNS3 is upregulated during osteogenic differentiation and downregulated during early adipogenesis. Silencing TNS3 promoted adipogenic differentiation (Oil red O staining: 4-fold; $p<0.001$) of BMSC at the expense of osteoblast differentiation (calcium assay: 3.5-fold; $p<0.001$). Actin polymerization was inhibited and cell morphological changes were affected during osteogenic induction. Conversely, induction of TNS3 enhanced osteoblast mineralization (calcium assay: 2-fold; $p<0.05$) and attenuated adipocyte formation (Oil red O staining: 1.2-fold; $p<0.01$). In an attempt to find mechanistic cues for the effect of TNS3 on differentiation, we came across the tumor suppressor Rho GTPase-activating protein 7 (DLC1), which was oppositely regulated by TNS3 during osteogenesis versus adipogenesis.

Conclusion(s): We identified TNS3 as a positive regulator in osteogenic differentiation and negative regulator in adipogenic

differentiation of BMSCs. It may hold the potential as a therapeutic target for treatment of osteoporosis.

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COP12

The inositol phosphatase SHIP1 regulates skeletal development

Fatemeh Safari[a,b], Jeremy Yeoh[b,c], Margaux Bringardner[a], Mark Siegrist[a], Silvia Dolder[a], Franziska Strunz[a,b], Willy Hofstetter[a], Philippe Krebs[c]
[a]*Bone Biology & Orthopaedic Research, Department for BioMedical Research DBMR- University of Bern, Bern, Switzerland*
[b]*Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland*
[c]*Institute of Pathology, University of Bern, Bern, Switzerland*

Background/Introduction: Src-homology (SH) 2 domain-containing inositol-5-phosphatase 1 (SHIP1) is a lipid phosphatase expressed mainly in hematopoietic cells. SHIP1 regulates cell proliferation, differentiation, and survival via the PI3K/Akt signaling pathway. SHIP1-deficient *(Styx)* mice are osteoporotic, which is associated with an increased number of osteoclasts (OC).

Purpose: This study aimed to investigate the underlying mechanisms through which SHIP1 controls osteoporosis.

Methods: Osteoclast progenitor cells (OPC) were generated by incubating bone marrow cells with CSF-1. To develop OC, OPC from *Styx*, *Styx het* (heterozygous) and *wt* (wild type) mice were cultured with RANKL and CSF-1. Osteoclastogenesis was evaluated using an XTT cell viability assay, TRAP activity (OC marker) and qRT-PCR. Micro-computed tomography (Micro-CT) of vertebrae and femora were performed to evaluate the bone structure.

Results: Deficiency in SHIP1 affected several aspects of bone. Compared to *Styx het* and *wt* controls, OPC-derived *Styx* OC presented several developmental defects, including a lower TRAP/XTT ratio and a 52% decrease in *Calcr* transcripts (encoding for the Calcitonin Receptor) ($p<0.001$). *In vivo*, there was a strong reduction of BV/TV in vertebrae and femora of *Styx* versus *wt* animals (39.6% and 35%, respectively, $p<0.01$). In particular, trabeculae in *Styx* vertebrae were increased by 8% ($p<0.05$) in numbers while decreased by 37% in thickness ($p<0.001$). In contrast, in *Styx* femora both the number and thickness of the trabeculae were decreased by 16% and 14%, respectively. These different phenotypes in *Styx* femora versus vertebrae indicate different paths to osteoporosis in bones with primary or secondary spongiosa.

Conclusion(s): Taken together, our data indicate a central role for SHIP1-dependent PI3K/Akt signalling in bone remodeling. Further investigation will address the role of osteoblasts in the development of osteoporosis in SHIP1-deficient *Styx* mice.

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Concurrent Oral Presentations 2: Clinical / Public Health: New Pathophysiology Insights

COP13

Epigenome-wide association study shows that smoking alters DNA methylation in blood cells triggering aggressive bone resorption of osteoclasts in vivo and in vitro

Salah Eddine Masmoudi[a], Anaïs M.J. Møller[b], Jonas S. Madsen[c], Jean-Marie Delaissé[b], Bjarne W. Rasnussen[d], Qihua Tan[a], Kent Søe[b]
[a]*Unit of Epidemiology- Biostatistics and Biodemography, Public Health-University of Southern Denmark, Odense, Denmark*
[b]*Clinical Cell Biology- Res. Unit of Pathology, Clinical Research-University of Southern Denmark, Odense, Denmark*
[c]*Lillebaelt Hospital- University Hospital of Southern Denmark, Biochemistry, Odense, Denmark*
[d]*Odense University Hospital, Pathology, Odense, Denmark*

Background/Introduction: Smoking is a risk factor for osteoporosis, but the mechanisms remain unclear. Human osteoclasts can resorb bone in two modes, the aggressive trench-mode and the slower pit-mode. Recently, we have shown[1] that e.g. donors' age correlate with osteoclast trench-mode *in vitro* suggesting an epigenetic regulation through monocytes.

Purpose: Investigate if smoking affects bone resorption of osteoclasts *in vivo* and *in vitro* and if this may be explained by changes in DNA methylation.

Methods: Based on data from our publication[1] we applied an epigenome-wide association study (EWAS)(n=34 healthy women, 40-66 years, ethical approval S-20150059). Methylation levels on DNA from donors' peripheral blood mononuclear cells were analysed using Illumina's EPIC array (850k CpGs). Osteoclasts generated from donors were reseeded onto bone slices for 72h and evaluated for % trench surface/eroded surface (%TS/ES).

Results: Number of cigarettes smoked throughout life (0 to 263,000) correlated with %TS/ES *in vitro* ($r_s=0.40$,$p=0.02$) and an increasing imbalance between bone resorption (CTX) and formation (PINP) *in vivo* (CTX/PINP) ($r_s=0.45$,$p=0.008$). A direct correlation between %TS/ES *in vitro* and CTX/PINP *in vivo* was also found ($r^2=0.20$,$p=0.009$). EWAS for single CpGs on%TS/ES interacting with number of cigarettes smoked throughout life showed a total of 2035CpGs with both positive and negative directions of significance associations between smoking and %TS/ES (FDR<0.05,p=9.88e-06). 2755CpGs were significant for CTX/PINP ratio *in vivo* interacting with the number of cigarettes smoked throughout life (FDR<0.05,p=4.703e-11). 1731CpGs were found overlapping in common.

Conclusion(s): Results suggest that smoking directly affects osteoclasts *in vivo* and *in vitro* triggering aggressive osteoclasts. This may be mediated through epigenome-wide alterations in circulating osteoclast precursors. Our data may be of interest to predict individual risk of osteoporosis, especially in the context of smoking history.

Reference: [1] Møller, A.M.J. et al.; Bone Res. 8, 27 (2020).

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COP14

Association between environmental air pollution and rheumatoid arthritis flares

Giovanni Adami, Davide Gatti, Angelo Fassio, Alessandro Giollo, Ombretta Viapiana, Maurizio Rossini
University of Verona, Rheumatology Unit, Verona, Italy

Background/Introduction: Environmental air pollution has been linked to the pathogenesis of Rheumatoid Arthritis (RA). Nevertheless, evidence linking higher concentrations of air pollutants with the risk of RA reactivations is missing.

Purpose: The objective of the study was to determine the association between RA flares and air pollution.

Methods: We designed a case-crossover study. We compared the exposure to pollutants in the 30-day and 60-day periods preceding an arthritic flare referent to the 30-day and 60-day preceding a low-

disease activity visit. Flare was defined as an increase in DAS28-CRP of >1.2 points with current DAS28-CRP ≥3.2.

Results: 888 patients with RA with 3,396 follow-up visits were included in the study. 13,636 daily air pollution records were retrieved. We found an exposure-response relationship between the concentration of air pollutants and the risk of having abnormal CRP levels (**figure 1**). 440 patients (49.5%) had at least 2 follow-up visits with a difference in DAS28-CRP of more than 1.2 points (with current DAS28-CRP ≥3.2), serving as our sample for the case-crossover study. Concentrations of CO, NO, NO2, NOx, PM10, PM2.5 and O3 were higher in the 60-day period preceding a flare (**table 1**).

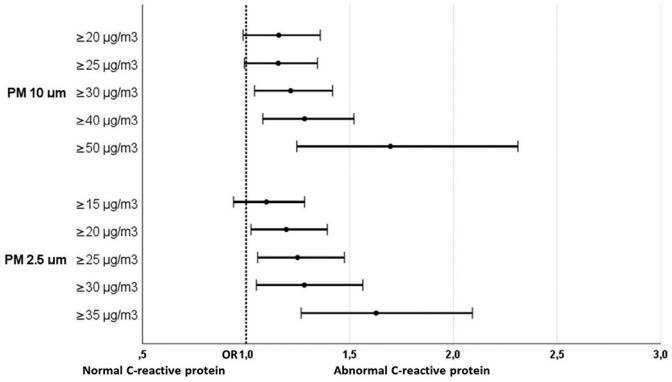

Figure 1. Odds of having abnormal CRP serum levels (≥5 mg/L)

Table 1
Area Under the Curve of air pollutants in the 60 days before low-disease activity visit and flare visit

Pollutant AUC ug/m3	Control period (n=440)	Flare period (n=440)	p value
CO	22.00	24.53	0.001
NO	1,120.53	1,403.88	0.002
NO2	1,800.96	1,892.05	0.040
NOx	3,515.77	4,041.06	0.004
PM10	1,789.22	1,942.52	0.005
O3	1,776.37	1,934.35	0.001
PM2.5	1,272.61	1,403.60	<0.001

Conclusion(s): We found a striking association between air pollution and RA disease severity and reactivations in a cohort of patients followed over a 5-year period. The exposure to high levels of air pollutants was associated with increased CRP levels and a higher risk of experiencing a flare of arthritis.

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COP15

In healthy men, early decline in trabecular bone mineral density is, in part, related to decreases in sex steroids

Thiberiu Banica, Charlotte Verroken, Hans-Georg Zmierczak, Stefan Goemaere, Jean-Marc Kaufman, Bruno Lapauw
Ghent University, Unit for Osteoporosis and Metabolic Bone Diseases- Department of Endocrinology- Ghent University Hospital, Ghent, Belgium

Background/Introduction: Bone mass is known to decline in aging men and this decline is in part affected by sex steroid exposure. However, it is unclear how early after achieving peak bone mass bone loss begins and whether this decline is associated with sex steroid levels in young adulthood.

Purpose: Investigating longitudinal changes in trabecular and cortical vBMD in relation to sex steroid levels, body composition and lifestyle factors in young adult men.

Methods: Longitudinal observational study. 999 healthy men aged 24-46 years of whom 691 were re-evaluated after a mean period of 12 years. Serum sex hormone binding globulin (SHBG) levels were measured using immuno-assay. Testosterone (T), estradiol (E2), were measured using LC-MS/MS, free T calculated (cFT). Volumetric BMD was determined at the non-dominant arm (radius, at 4% and 66% of bone length from distal) using pQCT (Stratec XCT-2000, Stratec Medizintechnik, Germany, version 6.0). Linear mixed models were used for statistical analyses. All models comprised lifestyle factors and were adjusted for age and body mass index (BMI).

Results: Baseline age was 34±6 years. Mean BMI increased by 1.19kg/m². Trabecular vBMD decreased by 1.7% (228.9mg/mm³ vs 225.0 mg/mm³), no changes over time in cortical vBMD were observed. Mean T levels decreased by 14.2% (20.8nmol/l vs. 17.8nmol/l), cFT by 19.1% (392pmol/l vs. 317pmol/l). Mean E2 levels did not change over time. SHBG increased by 3.0% (39.8nmol/l vs. 41.0nmol/l). Larger decreases in T, cFT and E2 (all $p<0.03$) but not SHBG ($p>0.05$) were associated with more pronounced decreases of trabecular vBMD over time.

Conclusion(s): Shortly after achieving peak bone mass, a modest trabecular decline was appreciated. This decline was in part associated with declining sex steroid levels. Moreover this decline persisted after correction for changes in body composition and lifestyle factors.

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COP16

Glucocorticoids prolong the reversal-resorption phase delaying bone formation in intracortical remodelling compared to postmenopausal and osteoporotic women

Lisbeth Koch Thomsen[a,b], Majken I. Kejser[a,b], Line L. Sørensen[a,b], Pia R. Jensen[c], Ellen-Margrethe Hauge[d], Jens Bollerslev[e,f], Lene W.T. Boel[g], Jean-Marie Delaissé[a,b], Christina M. Andreasen[a,b], Thomas Levin Andersen[a,b,g]
[a]*Odense University Hospital, Clinical Cell Biology- Department of Clinical Pathology, Odense, Denmark*
[b]*University of Southern Denmark, Research Unit of Pathology- Department of Clinical Research- Department of Molecular Medicine, Odense, Denmark*
[c]*University of Southern Denmark, Department of Clinical Cell Biology- Vejle Hospital- IRS, Vejle, Denmark*
[d]*Aarhus University Hospital, Department of Rheumatology, Aarhus, Denmark*
[e]*Oslo University Hospital, Section of Specialized Endocrinology- Medical Clinic B, Oslo, Norway*
[f]*University of Oslo, Faculty of Medicine, Oslo, Norway*
[g]*Aarhus University, Department of Forensic Medicine, Aarhus, Denmark*

Background/Introduction: Studies in women have demonstrated an age-related increased cortical porosity attributable to accumulation of eroded type 2 pores (remodeling of existing pores) reflecting an extended reversal-resorption phase.

Purpose: This study addresses the histomorphometric changes in cortical bone remodeling in glucocorticoid-induced osteoporosis (GIO) versus postmenopausal osteoporosis (PMO) and postmenopausal controls.

Methods: We analysed 7-µm-thick Goldner-Masson trichrome stained sections from transiliac bone biopsies from i) postmenopausal women with GIO (n=19, age 71±5 years), ii) women with PMO, without prior glucocorticoid-treatment (n=17, age 71±6 years), and iii) postmenopausal healthy women (controls, n=21, age 71±7 years).

Results: We found statistically significantly thinner cortices (~40%) in both GIO and PMO relative to controls ($p<0.001$), but only in controls the change was correlating with age ($r_p=-0.49$, $p<0.05$). In the cortex remaining, PMO had a borderline significantly decreased porosity compared to controls ($p=0.054$) and GIO ($p=0.066$). The porosity correlated with mean pore diameter (GIO: $r_p=0.84$, $p<0.0001$, PMO: $r_p=0.93$, $p<0.0001$, controls: $r_p=0.54$, $p<0.05$), but not pore density. The percentage of porosity reflecting non-quiescent pores was high in all three groups, although highest in GIO (89.2%, $p<0.05$) and PMO (88.2%, $p=0.051$) compared to controls (79.5%). In GIO, a significant higher percentage of the porosity was represented by eroded pores compared to PMO ($p<0.05$) and controls ($p<0.01$). Furthermore, in GIO a significantly lower percentage of the porosity reflected mixed eroded-formative pores than in PMO ($p<0.05$). The pore diameter of quiescent osteons was lower in GIO ($p=0.053$) and PMO ($p<0.001$) compared to controls, suggesting an improved BMU balance. No significant correlation with dosage or treatment-time was found in GIO, possibly due to the late stage in glucocorticoid-treatment at biopsy collection (6.0 ± 5.1 years).

Conclusion(s): In conclusion, GIO and PMO reveal a pronounced cortical thinning. Especially in GIO, the cortical porosity reflects cortical remodelling with an extended reversal-resorption phase – not a negative BMU balance.

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COP17

A whole genome sequencing study to identify novel genetic variants associated with lean mass: Multi-ethnic meta-analysis

David Karasik[a], Zhang Xiaoyu[b], Su Kuan-Jui[c], Liu Ching-Ti[b], Xu Hanfei[b], Fu Mao[d], Wiggins Kerri[e], Hsu Yi-Hsiang[f], Yau Michelle[f], Crandall Carolyn[g], Mitchell Braxton[d], Jackson Rebecca[h], Montasser May[d], P. Kiel Douglas[f], Deng HongWen[c]

[a]*Azrieli Faculty of Medicine, Bar-Ilan University, Safed, Israel*
[b]*Biostatistics, Boston University School of Public Health, Boston, USA*
[c]*Department of Medicine, Tulane University, New Orleans, USA*
[d]*School of Medicine, University of Maryland, Baltimore, USA*
[e]*Cardiovascular Health, University of Washington, Seattle, USA*
[f]*Marcus Institute for Aging Research, Hebrew SeniorLife, Boston, USA*
[g]*Health Sciences, University of California, Los Angeles, USA*
[h]*School of Medicine, The Ohio State University, Columbus, USA*

Background/Introduction: Genome-wide association studies (GWAS) of whole-body (WB-) and appendicular (a-) LM are unlikely to identify rare variations that may have larger effect sizes than common variants.

Purpose: A whole-genome sequencing (WGS) study was performed to comprehensively discover sequence variants associated with variation in DXA-derived lean mass (LM), a proxy of muscular fitness.

Methods: We utilized deep-coverage WGS (average 30x) in ethnically diverse samples from the Trans-Omics for Precision Medicine (TOPMed) program (n=5744; 85% European-Ancestry, 7% African-Ancestry, 8% Hispanic /Latino); TOPMed participants had a mean age of 60.7 years (SD: 14.8) and >65% of participants were women. We then meta-analyzed results with the Louisiana Osteoporosis Study (LOS), n~5000, who included 58% European, 42% African ancestry, ~50% men, with a mean age of 39.2 years (SD: 11.2). Lean mass residuals were generated adjusting for age, age^2, sex, height, weight, total fat and study-specific variables. Genetic associations with inverse normal transformed residuals were evaluated using linear mixed-effects models. To meta-analyze variants with minor allele frequency (MAF) ≥ 0.1%, we used a fixed effects approach.

Results: In the single-variant analysis, several rare variants with MAF <1% were significantly ($p<5*10(-8)$) associated with WB LM: rs116652927, rs148735123, and rs77796060; while rs182466396 (MAF=1.5%) was associated with aLM. The latter is an intronic variant in *SSUH2*, a gene associated with rippling muscle disease 2, which is a form of limb-girdle muscular dystrophy. There were no known muscle GWAS signals within 1 Mb from these variants.

Conclusion(s): Additional analyses are underway, including sex- and ancestry-specific analyses. The discovery of novel genetic variants associated with lean mass may provide new insights into pathways influencing muscle metabolism and muscle mass regulation.

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COP18

HR-pQCT-based regional analysis reveals tibial spatial variability in cortical bone tissue quality in type 2 diabetic postmenopausal women with and without history of fragility fractures

Ursula Heilmeier[a], Courtney Pasco[b], Meliss Yilmaz[c], Andrew J. Burghardt[d], Gabby B. Joseph[d], Thomas M. Link[d], Galateia J. Kazakia[d]

[a]*ECTS Academy, Musculoskeletal Quantitative Imaging Research Group-University of California San Francisco, Zurich, Switzerland*
[b]*University of California Berkeley, Department of Bioengineering, Berkeley, United States*
[c]*University of California Berkeley, Department of Molecular and Cell Biology, Berkeley, United States*
[d]*University of California San Francisco, Musculoskeletal Quantitative Imaging Research Group, San Francisco, United States*

Background/Introduction: Type 2 diabetic (T2D) bone disease is characterized by an increased fracture risk which is partly ascribed to cortical deficits such as higher cortical porosity. While previous work identified midcortical and periosteal layers as potential high-porosity zones in T2D, the anatomical spatial variability of cortical porosity in T2D remains unknown.

Purpose: Using high-resolution peripheral quantitative computed tomography (HR-pQCT), we therefore aimed to characterize and compare the regional variability of cortical porosity and other cortical measures in T2D women with (DMFx) and without history of fragility fractures (DM).

Methods: 39 postmenopausal women (n=20 DMFx, n=19 DM) underwent HR-pQCT scanning of the distal tibia. Cortical parameters including cortical porosity measures were calculated for the overall tibial cortex and for the anterior, posterior, medial and lateral cortical quadrants which were defined based on anatomic axes.

Results: Using linear regression models we found that DMFx subjects exhibited significantly higher global macro-porosity (Ct.Po) than DM subjects (+128%, p=0.001), but also significantly higher regional macro-porosity in each of the four tibial quadrants (+94% to +134%, $0.033\leq p\leq 0.001$). In all but the anterior quadrant, significantly larger pores (Ct.Po.Dm: + 17% to +21%, $0.025\leq p\leq 0.003$), and larger pore heterogeneity (Ct.Po.Dm.SD, +24%

to +33%, 0.022≤p≤0.002) were additionally noted in DMFx vs DM subjects. Furthermore, the posterior tibial cortex of DMFx subjects displayed significantly lower cortical tissue mineral density (Ct. TMD) – a bone tissue quality marker encompassing tissue mineralization and sub-resolution micro-porosity – and thus significantly poorer bone tissue quality (p=0.022) compared to DM subjects. All other quadrants lacked significant Ct.TMD differences between groups.

Conclusion(s): Using regional analysis, we found significantly poorer bone tissue quality in the posterior tibial cortex in T2D women with than without history of fragility fractures. As this area undergoes peak compressive strains during walking, the poorer bone tissue quality may facilitate microcrack formation and propagation and thus contribute to diabetic bone fragility.

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Concurrent Oral Presentations 2: Basic / Translational: Animal Models and Bone Diseases

COP19

Monitoring musculoskeletal health in zebrafish using machine learning-based locomotion tracking

Imke Astrid Kristina Fiedler[a], Martin Gromniak[b], Debayan Bhattacharya[b], Alexander Schlaefer[b], Björn Busse[a]
[a]*University Medical Center Hamburg-Eppendorf, Department of Osteology and Biomechanics, Hamburg, Germany*
[b]*Hamburg University of Technology, Institute of Medical Technology and Intelligent Systems, Hamburg, Germany*

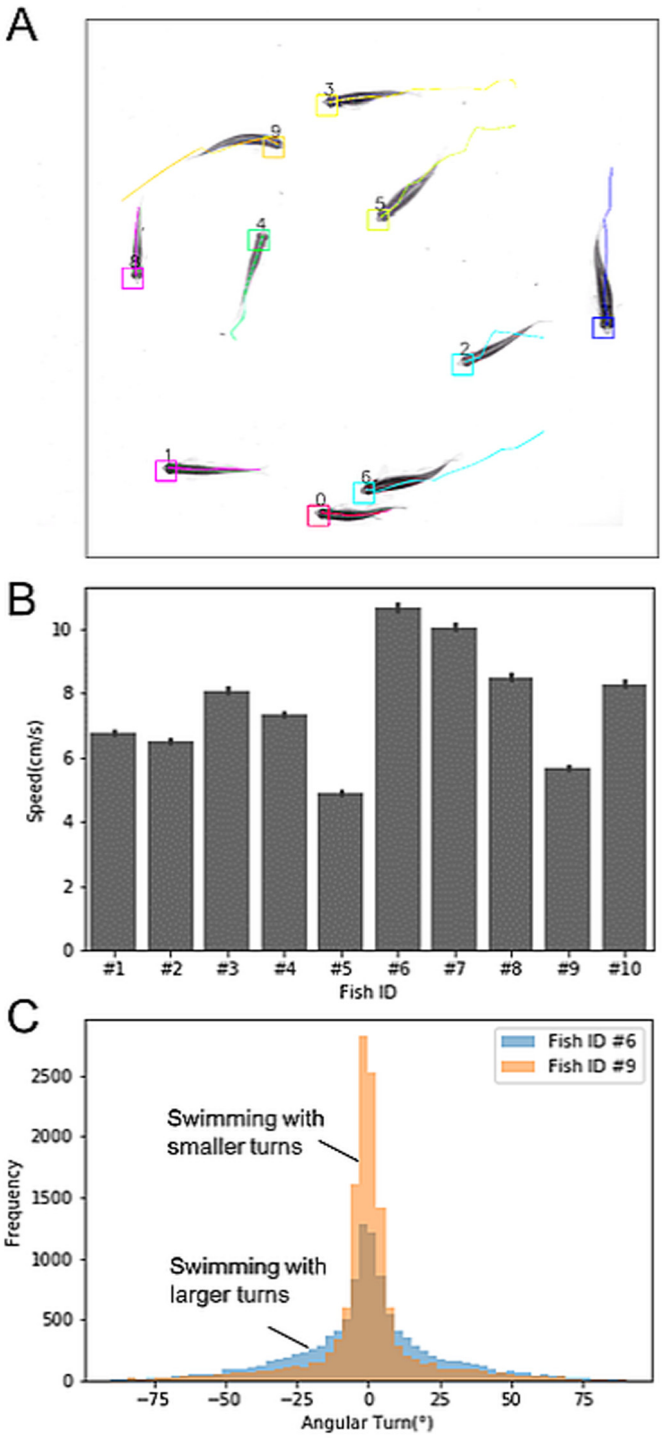
Background/Introduction: Similar to humans, the physical mobility of zebrafish can be impaired due to aging or disease (e.g. osteoarthritis, scoliosis). While pathological locomotion patterns of individual fish may appear obvious to the observer, visual inspection remains difficult because of multiple moving objects, rendering the quantification of changes in mobility impossible.

Purpose: Hypothesizing that swimming patterns serve as mobility markers, we propose a machine learning-based approach to identify individual fish in a group and monitor their physical activity.

Methods: The set-up consisted of an aquarium tank (water volume 30x30x10cm), a top and side view camera. Adult wild-type zebrafish (N=10) were recorded. Top view video frames of single fish were used to train the neural network algorithm for recognizing each fish based on its morphological head characteristics. Video frames of the entire zebrafish group swimming in the tank were acquired for 15min. Within these frames, individual fish were identified and their time-stamped trajectories were generated.

Results: The classification algorithm (accuracy of 99.6%) and tracking algorithm (precision and recall of 96.2 %) were sufficiently robust to detect and track individual fish within the group (Fig. 1A). We quantified the total swimming distance (m), average swimming speed (cm/s), and angular turns (°) (Fig. 1B-C). Large variability was found even between fish of the same age group with identical housing conditions.

Conclusion(s): This study is the first step towards *in vivo* assessment of physical performance, locomotion phenotypes, and treatment strategies in zebrafish models of skeletal disease, yielding an enormous potential in the scope of preclinical studies in the musculoskeletal field.



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COP20

Circulating microRNA analysis in ZDF rats as a model for the identification of biomarkers and disease mechanisms in diabetic osteopathy

David Carro Vázquez[a], Christine Hofbauer[b], Lejla Emini[b], Martina Rauner[b], Johannes Grillari[c], Richard Eastell[d], Lorenz Hofbauer[b], Matthias Hackl[a]
[a]*Tamirna GmbH, Department of Biomarker Development, Wien, Austria*
[b]*Bone Lab, Department of Medicine III and Center for Healthy Aging-Technische Universität Dresden, Dresden, Germany*

[c]*Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Department of Experimental and Clinical Traumatology, Vienna, Austria*
[d]*Mellanby Centre for Bone Research, Department of Oncology and Metabolism, Sheffield, United Kingdom*

Background/Introduction: Metabolic changes in Type-2 Diabetes-Mellitus (T2DM) make patients more prone to develop osteoporosis and delayed bone healing. We hypothesize that microRNAs could be involved in the underlying mechanism and used as biomarkers in this context.

Purpose: To test this hypothesis, we analyzed microRNAs in samples from Zucker Diabetic Fatty (ZDF) rats, a T2DM model with reduced bone healing and bone mass.

Methods: Serum and ulna samples of ZDF and wildtype rats with and without an induction of bone healing by a femur subcritical defect were obtained (n=5-6). One positive control (miR-122) as well as five microRNA candidates were analyzed by RT-qPCR. ANOVA analysis was applied to test for differential expression ($p<0.05$). Pearson correlation analysis was performed to identify associations between the microRNA levels and bone phenotypic data ($p<0.05$).

Results: Significant increases ($p<0.05$) of miR-122-5p serum levels, a liver-enriched microRNA associated with T2DM, were found in ZDF rats confirming previous results. Amongst our candidates circulating miR-375 showed the strongest up-regulation in diabetic rats independent of whether femur was intact or defect. Similar trends in expression were observed in ulna samples (Figure 1), and miR-375 serum levels were inversely correlated ($p<0.05$) to bone structural parameters, including the total density in vertebra, the trabecular density in femur and the BV/TV in tibia.

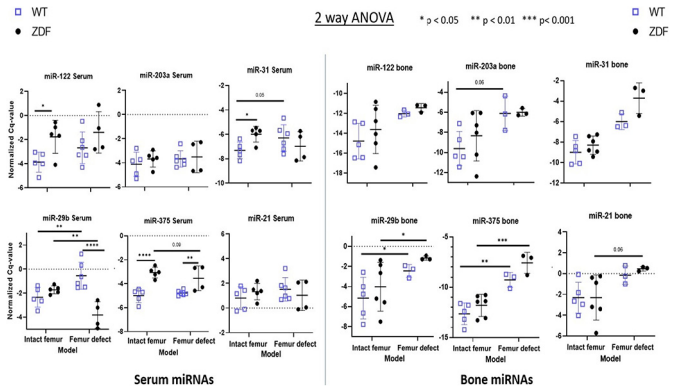

Figure 1.

Conclusion(s): Levels of miR-375, a microRNA known to inhibit osteogenesis by negative regulation of RUNX2 in hMSC and of LRP5 and β-catenin in MC3T3-E1 cells, were found differentially regulated in serum and bone of diabetic rats. Correlation analysis suggest a relation between decreased bone quality in the context of diabetes and microRNA expression. Further studies need to be conducted to assess the causality behind this correlation.

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COP21

Alcoholic liver disease-induced changes in microstructural and mechanical properties of the femoral neck: An autopsy study

Jelena Jadzic[a], Petar Milovanovic[a], Danica Cvetkovic[b], Miomira Ivovic[c], Nada Tomanovic[d], Vladimir Zivkovic[b], Slobodan Nikolic[b], Marija Djuric[a], Danijela Djonic[a]
[a]*Laboratory For Anthropology And Skeletal Biology- Institute For Anatomy, Faculty Of Medicine- University Of Belgrade, Belgrade, Serbia*
[b]*Institute of Forensic Medicine, Faculty of Medicine- University of Belgrade, Belgrade, Serbia*
[c]*Institute for Endocrinology- Diabetes and Metabolic Diseases, Clinical Center of Serbia, Belgrade, Serbia*
[d]*Institute of Pathology, Faculty of Medicine- University of Belgrade, Belgrade, Serbia*

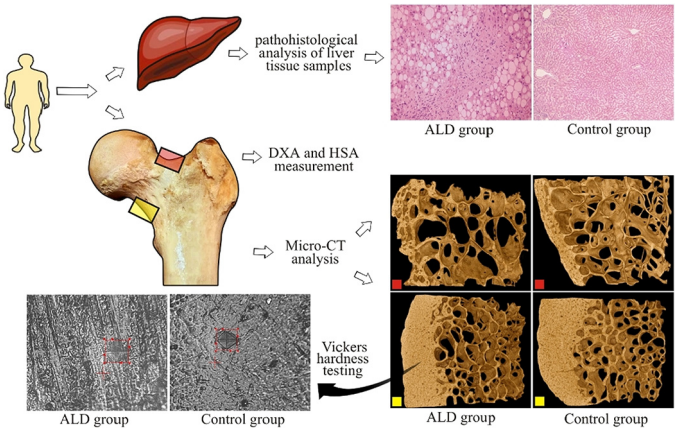
Background/Introduction: Recent studies showed an increased hip fracture risk in patients with alcoholic liver disease (ALD), while their femoral mechanostructural characteristics were not investigated previously.

Purpose: This study aimed to examine the difference in clinical imaging findings, microstructural and mechanical properties of the femoral neck sampled from ALD donors (n=13, age:57±13 years) and healthy sex- and age-matched controls (n=20, age:54±13 years). The Institutional Ethics Committee approved sample collection from cadaveric donors.

Methods: Following patho-histological verification of ALD, DXA and HSA measurement were performed in combination with micro-CT and Vickers microhardness analysis of superolateral and inferomedial femoral neck (Figure 1).

Results: Densitometric and HSA parameters were not significantly declined in femoral neck of ALD donors ($p>0.05$ after BMI adjustment). However, significant deterioration of trabecular and cortical microstructure was noted in superolateral (BV/TV: 14.3±2.7% vs. 20.5±3.5%, Tb.N 0.84±0.12/mm vs. 1.03±0.10/mm; Ct.Po: 26.7±8.1% vs. 17.8±5.7%, Ct.Th: 300.7±68.6μm vs. 322.1±62.2μm, Po.Dm 0.242±0.061mm vs. 0.109±0.105mm) and inferomedial femoral neck (BV/TV: 19.2±3.4% vs. 22.7±5.8%, Tb.N 0.92±0.14 /mm vs. 0.98±0.20/mm; Ct.Po:14.2±6.2% vs. 8.2±2.6%, Ct.Th: 493.1±196.1μm vs. 619.5±129.8μm, Po.Dm: 0.208±0.043mm vs. 0.177±0.043mm) of ALD donors in comparison to control group ($p<0.05$). Additionally, reduced cortical Vickers hardness values of superolateral (61.6±4.8VH vs. 75.1±5.8VH, $p<0.001$) and inferomedial neck sample (62.7±5.8VH vs. 74.6±6.5VH, $p<0.001$) were noted in cadaveric donors with ALD (Figure 1).

Conclusion(s): In conclusion, microstructural alteration and Vickers hardness changes observed in our study may elucidate the morphological basis for increased bone fragility and hip fracture risk



in ALD patients. Thus, the evaluation of hip fracture risk should be thoroughly performed in ALD patients.

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COP22

Atypical type VI Osteogenesis Imperfecta mouse models the intersection of IFITM5 and SERPINF1 pathways in patients

Gali Guterman-Ram[a], Ghazal Hedjazi[b], Chris Stephan[c], Stéphane Blouin[b], Jochen Zwerina[b], Kenneth M. Kozloff[c], Nadja Fratzl-Zelman[b], Joan C. Marini[a]
[a]*Eunice Kennedy Shriver National Institute of Child Health and Human Development- National Institutes of Health, Section on Heritable Disorders of Bone and Extracellular Matrix, Bethesda, United States*
[b]*Hanusch Hospital of OEGK and AUVA Trauma Centre Meidling- 1st Med. Dept. Hanusch Hospital, Ludwig Boltzmann Institute of Osteology, Vienna, Austria*
[c]*University of Michigan, Departments of Orthopaedic Surgery and Biomedical Engineering, Ann Arbor, United States*

Background/Introduction: 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 mutation leads to atypical type-VI OI (aVI), reported in 9 patients, causes severe dominant OI with phenotype, bone histology and decreased cellular secretion of PEDF similar to type VI OI, rather than Type V OI.

Purpose: Our objective is understanding the role of the pathways connecting *IFITM5* and *SERPINF1* in bone development.

Methods: We generated an *Ifitm5* S42L knock-in mouse model with NICHD-ACUC research approval.

Results: Newborn *Ifitm5* S42L mice, both heterozygous and homozygous, are non-lethal, have flared rib cage, shoulder and knee dislocations, while homozygotes have rib fractures and unmineralized calvaria. Radiographically, heterozygous mice exhibit »50% humeral fractures at multiple ages, while homozygotes incur fractures in 96% of humerii, femora and pelvis. Similar to aVI OI patients, young heterozygous males have normal PEDF level and increased serum ALP($p<0.01$). Mechanical testing of 2-month heterozygous and homozygous males showed reduced stiffness, yield and ultimate load, with markedly increased brittleness. Vascular pore volume/BV was increased in heterozygous males on μCT; serum Vegf levels were significantly decreased in heterozygous females. Whole-body DXA-aBMD was significantly decreased in 1,2-and 6-month-old mice with a step effect, suggesting homozygotes are more severe than heterozygotes ($p<0.01$). Preliminary data indicate increased osteoid perimeter to bone perimeter($p<0.05$) in heterozygous males. qBEI revealed hypermineralization in 1-and 2-month heterozygous vs. WT males, with increased CaMean, CaPeak($p<0.05$) and tripling of CaHigh in cortical bone($p<0.0001$ in 1-month-old,$p=0.0027$ in 2-month-old mice). Cultured calvarial and long bone osteoblasts exhibit differences in differentiation pattern, dependent on mating scheme, age and skeletal site.

Conclusion(s): 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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COP23

Novel murine model of atypical type VI osteogenesis imperfecta has altered osteocyte canalicular network, disordered collagen orientation along with hypermineralization of bone matrix

Ghazal Hedjazi[a], Gali Guterman-Ram[b], Stéphane Blouin[a], Markus A. Hartmann[a], Victoria Schemenz[c], Wolfgang Wagermaier[c], Peter Fratzl[c], Jochen Zwerina[a], Nadja Fratzl-Zelman[a], Joan C. Marini[b]
[a]*Ludwig Boltzmann Institute of Osteology, Hanusch Hospital of OEGK and AUVA Trauma Centre- Meidling-1st Med. Department Hanusch Hospital, Vienna, Austria*
[b]*NIH, Section on Heritable Disorders of Bone and Extracellular Matrix-NICHD, Bethesda, United States*
[c]*Max Planck Institute of Colloids and Interfaces, Dept. of Biomaterials, Potsdam, Germany*

Background/Introduction: Atypical type VI Osteogenesis imperfecta (OI), reported only in 9 patients with extremely severe bone fragility phenotype, results from loss-of-function mutation (p. S40L) in *IFITM5/BRIL*, the causative gene of OI type V and decreased cellular PEDF secretion as in OI type VI.

Purpose: Therefore, to obtain information at bone tissue level and micrometer scale, we characterize bone material properties in a novel heterozygous *Ifitm5*S42L knock-in (KI) murine model at 4- and 8-weeks of age.

Methods: Cortical femoral bone from female wild-type (WT) and KI (n=10 per age and genotype) were analyzed by quantitative backscattered electron imaging, micro-CT, second harmonic generation fluorescence microscopy and confocal laser scanning microscopy, to assess bone mineralization density distribution, osteocyte lacunae sections (OLS), vascularization, collagen orientation and osteocyte canalicular density. Effects of age and genotype were tested by two-way ANOVA.

Results: We observed increased matrix mineralization in older versus younger animals, for each genotype, (CaPeak WT: +8.3%, KI: +6.85%, both $P<0.0001$), as well as decreased OLS density (WT: -27.2%, $P=0.026$; KI: -35.7%, $P<0.0001$) and decreased vascular pore number (WT: -27.6%, KI: -32.3%, both $P<0.0001$). Neither collagen orientation nor canalicular density were significantly altered by aging. In comparison to WT, KI bone showed increased CaPeak (4-week: +5.7%, 8-week: +4.2%, both $P<0.0001$), increased OLS density (4-week: +40.1%, $P=0.0008$), decreased osteocyte canalicular density (n=20, 4/8-week pooled: -14.7%, $P<0.0004$), increased vascular pore number (4-week: +10.8%, $P=0.006$) and increased relative amount of disordered matrix (4-week: +155%, 8-week: +170%, both $P<0.0001$).

Conclusion(s): The mouse model exhibitstypical OI bone material properties, such as elevated matrix mineralization and increased number of osteocyte lacunae and, as in OI type VI, increased vascularity. The observed reduction in osteocyte canalicular density might impact mechanosensing ability of the bone tissue. In addition to disordered collagen, these abnormalities contribute to deteriorating bone mechanical properties in this mouse model and most likely also in affected patients.

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COP24

Non-genotoxic conditioning to overcome transplant-related toxicity in oc/oc osteopetrotic mouse model

Valentina Capo[a,b], Sara Penna[a,c], Ludovica Santi[a], Andrea Cappelleri[d,e], Stefano Mantero[b,f], Eugenio Scanziani[d,e], Anna Villa[a,b]
[a]*IRCSS San Raffaele Scientific Institute, San Raffaele Telethon Institute for Gene Therapy, Milan, Italy*

[b]CNR-IRGB, Milan Unit, Milan, Italy
[c]University of Milano-Bicocca, Dimet, Monza, Italy
[d]University of Milan, Department of Veterinary Medicine, Milan, Italy
[e]University of Milan, Mouse and Animal Pathology Lab MAPLab Fondazione UniMi, Milan, Italy
[f]Humanitas Clinical and Research Center - IRCSS, n, Rozzano, Italy

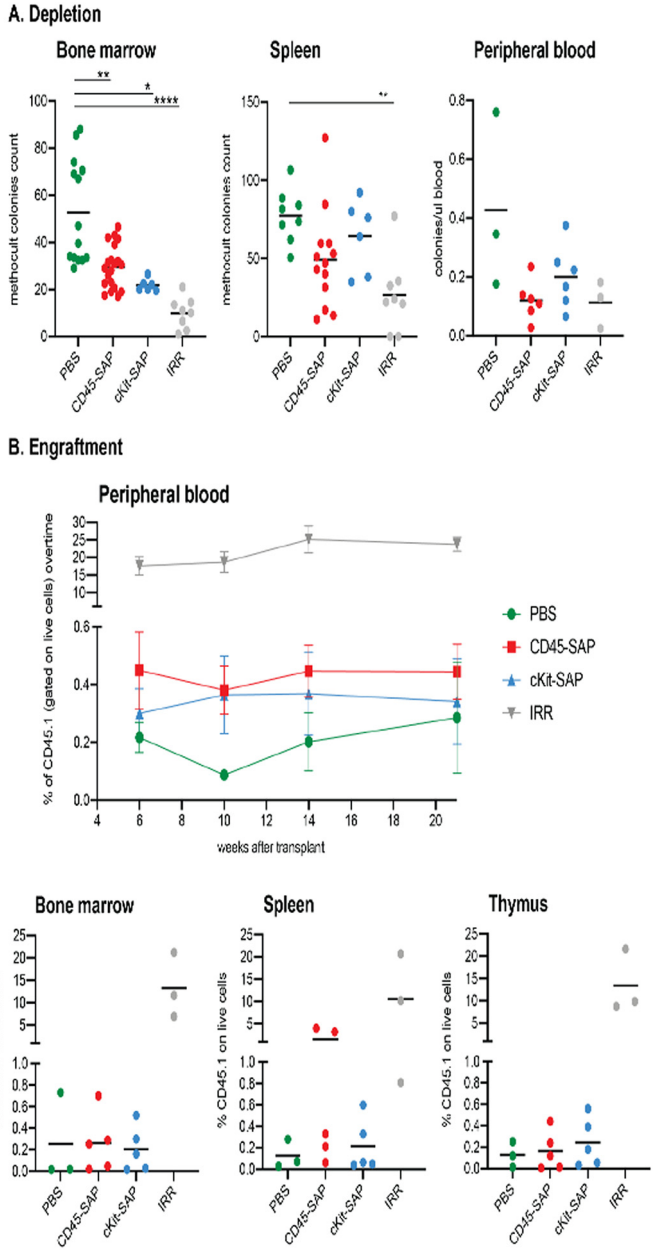
Background/Introduction: Autosomal recessive osteopetrosis (ARO) is a rare disease, affecting osteoclast differentiation or function. Most patients present mutations in *TCIRG1* gene, encoding a proton pump necessary for bone resorption. Symptoms include dense and brittle bones, limited bone marrow cavity, anaemia and progressive nerve compression, leading to death in the first decade of life. Standard treatment with allogeneic hematopoietic stem cell transplantation is hampered by HLA-matched donor availability, conditioning toxicity and significant morbidity

Purpose: Our aim is to test the efficacy of non-genotoxic conditioning in the osteopetrotic *oc/oc* murine model, to reduce transplant-related complications and mortality while ensuring efficient engraftment of donor cells.

Methods: Saporin (SAP) toxin was conjugated to anti-CD45 or anti-cKit antibodies to obtain CD45-SAP or cKit-SAP antibody-drug conjugates (ADCs). Due to the limited lifespan of *oc/oc* mice, we set up experimental conditions in WT newborn mice, that were conditioned with ADCs and transplanted with WT Lin- cells after 2 days. Survival and engraftment were monitored overtime. Animal procedures were approved by Institutional Animal Care and Use Committee.

Results: We observed partial HSPC (hematopoietic stem and progenitor cell) depletion with both CD45-SAP and cKit-SAP ($p<0.05$ and $p<0.01$ vs untransplanted controls, respectively) (Figure 1A). Accordingly, peripheral blood showed a modest increase of engraftment in SAP-conditioned and transplanted mice compared to untreated controls. Similar results were obtained in hematopoietic organs at sacrifice 4 months after transplant (Figure 1B).

Conclusion(s): ADCs can achieve sufficient HSPC depletion in newborn mice. However, further work to optimize engraftment efficacy in osteopetrotic setting is required.



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Concurrent Oral Presentations 3: Clinical / Public Health: Osteoporosis and Fractures

COP25

Persistence with osteoporosis treatment in patients from the Lille University Hospital Fracture Liaison Service

Anthony Delbar[a], Pflimlin Arnaud[a], Isabelle Delabrière[b], Camille Ternynck[c], Chantelot Christophe[d], François Puisieux[b], Bernard Cortet[a], Julien Paccou[a]
[a]Lille University Hospital, Rheumatology, Lille, France
[b]Lille University Hospital, Department of Gerontology, Lille, France
[c]Lille University Hospital, ULR 2694 - METRICS: Évaluation des technologies de santé et des pratiques médicales, Lille, France
[d]Lille University Hospital, Department of Traumatology, Lille, France

Background/Introduction: A Fracture Liaison Service (FLS) was set up in 2016. Data on persistence with osteoporosis medication were not available.

Purpose: The purpose was to assess persistence with osteoporosis treatment in patients from the FLS over a period of 1 year, and to determine predictors of discontinuation.

Methods: The study population comprised adults, aged 50 or over, admitted between 2016 and 2019 for a low-trauma fracture and managed in our FLS. Outcomes included (1) persistence rate at 1 year after treatment initiation, (2) persistence rate at 2 years, (3) persistence rate at 1 and 2 years according to type of treatment, (4) predictors of non-persistence. Persistence was determined using the Kaplan–Meier method.

Results: 1,224 patients were identified. 380 patients – 79.2% female; mean (SD) age 76 (11) years – were seen at the FLS. 410 fractures were found: vertebra (44%), hip (19%), and proximal humerus (10%). Osteoporosis treatment was prescribed for 367 (96.6%) patients and 275 of them began the prescribed treatment. The following drugs were prescribed: zoledronic acid (ZOL n=150, 54.5%), teriparatide (n=63, 22.9%), denosumab (n=39, 14.2%), and oral bisphosphonates (n=23, 8.4%). Persistence (any class) was estimated at 84.1% (95% CI: 79.1% to 88.1%) at 12-month follow-up, and dropped to 70.3% (63.7% to 75.9%) at 24 months. When drug-specific analyses were performed, persistence rates at 12 and 24

months were found to be higher with denosumab. Independent predictors of non-persistence at 12 months were 'follow-up performed by a general practitioner (GP)' – Odds Ratio (OR) for GP *vs.* FLS = 3.68; 1.52 to 8.90, p=0.004 – and 'treatment with ZOL' – OR for ZOL *vs.* denosumab = 3.39; 1.21 to 9.50, p=0.019; OR for ZOL *vs.* teriparatide = 8.86; 1.15 to 68.10, p=0.035.

Conclusion(s): This study provides evidence of the success of our FLS in terms of long-term persistence with injectable osteoporosis treatments.

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COP26

MOF/Hip fracture ratio in a Belgian cohort of post-menopausal women (FRISBEE): Potential impact on the FRAX® score

Aude Mugisha[a], Pierre Bergmann[b], Virginie Kinnard[c], Laura Iconaru[d], Felicia Baleanu[d], Alexia Charles[d], Murielle Surquin[a], Rafik Karmali[d], Florence Benoit[a], Jean-Jacques Body[e]
[a]*CHU Brugmann, Internal Medicine, Bruxelles, Belgium*
[b]*CHU Brugmann, Nuclear Medicine- Translational Medicine, Bruxelles, Belgium*
[c]*CHU Brugmann, Nuclear Medicine, Bruxelles, Belgium*
[d]*CHU Brugmann, Endocrinology, Bruxelles, Belgium*
[e]*CHU Brugmann, Internal Medicine- Endocrinology- Translational Medicine, Bruxelles, Belgium*

Background/Introduction: The ratio between major osteoporotic fractures (MOFs) and hip fractures in the Belgian FRAX® predictive tool is currently based on Swedish data.

Purpose: We studied the fracture distribution in a prospective cohort of Belgian postmenopausal women.

Methods: 3560 women aged 60 to 85 years (70.1±6.4 years), were included in a prospective study from 2007 to 2013 and surveyed yearly (FRISBEE). We analyzed the number of validated incident fractures according to age and sites and compared the MOFs/hip ratios in the FRISBEE cohort with the Swedish database.

Results: We registered 1336 fractures after a mean follow-up of 9.1 years. Compared to the Swedish data, the MOFs/hip ratios were systematically higher, by a factor of 1.7-1.8, in the FRISBEE cohort for all age groups. The MOFs/hip ratios for the FRISBEE cohort were 10.7 (95% CI: [5.6-20.5]), 6.4 [4.7-8.7] and 5.2 [3.9-6.5] for women 60-69, 70-79 and 80-89 years old, respectively. In the Swedish database, these ratios decreased from 4.8 for the 60-64 age group down to 1.5 for the 85-89 age group. The overall MOFs/hip ratio in our cohort was 6.0 [5.9-6.1]), which was higher than any Swedish ratio between 65 and 85 years. Nevertheless, the decrease of the ratios with age was parallel in the FRISBEE cohort to that observed in Sweden.

Conclusion(s): In our large prospective cohort, MOFs/hip ratios were 1.7-1.8 times those observed in the Swedish population used for fracture prediction in the Belgian version of FRAX®. This can seriously impact the estimation of the risk of MOFs, which is among the main criteria used to recommend a pharmacological treatment for osteoporosis in several countries. Our data suggest that the Belgian FRAX® for MOFs should be re-calibrated, and that similar validation studies could be conducted in other countries, which use the same Swedish MOF/hip ratio for MOFs prediction.

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COP27

Poor trabecular microarchitecture is associated with higher fracture risk in men followed for 12 years – the prospective STRAMBO study

Pawel Szulc[a], Dominique Foesser[b], Roland Chapurlat[c]
[a]*INSERM UMR 1033, Epidemiology of Osteoporosis, Lyon, France*
[b]*INSERM Unit 1033, Epidemiology of Osteoporosis, Lyon, France*
[c]*INSERM Unit 1033, Rheumatology, Lyon, France*

Background/Introduction: Poor bone microarchitecture assessed by high-resolution peripheral quantitative computed tomography (HR-pQCT) is associated with high fracture risk in the mid-term perspective. However, long-term utility of HR-pQCT indices for fracture prediction in older men has been poorly studied.

Purpose: Our aim was to study fracture prediction by bone microarchitectural indices in a cohort of men followed prospectively for 12 years.

Methods: Among 825 men aged 60–87, 160 men had fragility fractures, 34 had multiple fractures and 268 men died. Bone microarchitecture was assessed by HR-pQCT (XtremeCT SCANCO, SCANCO, Switzerland).

Results: After adjustment for age, BMI, femoral neck bone mineral density (BMD), prior fractures and falls, low trabecular number (Tb.N) at distal radius was associated with higher fracture risk (HR=1.45 per SD decrease, 95%CI: 1.21–1.73, p<0.001). Fracture risk was higher in the lowest vs. the highest quintile of Tb.N (HR=1.91, 95%CI: 1.08–3.36, p<0.05). Higher intra-individual trabecular distribution (Tb.1/N.SD) at distal radius was also associated with higher fracture risk (HR=1.41 per SD, 95%CI: 1.15–1.72, p<0.001). The risk was higher in the highest vs. the lowest quintile (HR=2.21, 95%CI: 1.21–4.01, p<0.01). The pattern was similar for multiple fractures (Tb.N: HR=1.76 per SD decrease, 95%CI: 1.19–2.59, p<0.005). At distal tibia, higher Tb.1/N.SD was associated with higher risk of all (HR=1.61 per SD, 95%CI: 1.03–2.49, p<0.05) and multiple fractures (HR=1.51, 95%CI: 1.06–2.16, p<0.01). The links of other indices with the fracture risk was weak (e.g., trabecular BMD) or non-significant (cortical measures, m-finite element indices). The patterns persisted after accounting for competing risk of death, e.g., lower distal radius Tb.N remained associated with higher risk of all (HR=1.45 per SD decrease, 95%CI: 1.21–1.73, p<0.001) and multiple fractures (HR=1.61 per SD decrease, 95%CI: 1.08–1.63, p<0.01).

Conclusion(s): Overall, poor trabecular microarchitecture as assessed by HR-pQCT is associated with a long-term increase in fracture risk in older men followed prospectively for 12 years.

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COP28

Incidence and risk factors for morphometric vertebral fractures in Japanese men and women: The ROAD study 3rd to 5th surveys

Chiaki Horii[a], Toshiko Iidaka[b], Yasushi Oshima[a], Sakae Tanaka[a], Noriko Yoshimura[b]
[a]*The University of Tokyo Hospital, Department of Orthopaedic Surgery, Tokyo, Japan*
[b]*The University of Tokyo Hospital, Department of Preventive Medicine for Locomotive Organ Disorders- 22nd Century Medical & Research Center, Tokyo, Japan*

Background/Introduction: Vertebral fractures (VFs) are the most common osteoporotic fractures; reports on their epidemiology remain limited.

Purpose: To estimate the incidence and risk factors of morphometric VFs in Japanese men and women.

Methods: Data from a population-based cohort study, the Research on Osteoarthritis/Osteoporosis Against Disability (ROAD) study, were analysed. Of the participants of the 3rd survey (2012–13, the baseline of the present study) with whole-spine radiography, 1,544 individuals (506 men and 1,038 women, mean age: 65.6 years) comprised the present study cohort, who were followed up for 6 years in the 4th (2015–16) and 5th (2018–19) surveys. Genant's semiquantitative method (SQ) was used to define VF: SQ=1, mild deformity (MD); SQ ≥2, VF. Incident VFs were defined when at least one vertebra with SQ <2 at baseline was diagnosed as a VF at either of the follow-ups. The incidence of VF was calculated using the person-year method. The Cox proportional hazard model was used to assess risk factors for incident VFs.

Results: The prevalence of VFs at baseline was 7.6% (4.7% in men, 9.1% in women). Twenty men and 91 women sustained incident VFs in 6 years. The incidence of VF was 14.5 (8.0 in men, 17.7 in women)/1,000 person-years. The significant risk factors for incident VFs were age, female sex, the presence of MD at baseline, and the presence of VF at baseline [hazard ratios (HRs), 95% confidence intervals (CIs): 1.07, 1.04–1.09; 2.21, 1.20–4.07; 5.69, 3.53–9.18; 6.48, 3.76–11.16, respectively]. Furthermore, advanced age of menarche was a significant risk factor for incident VFs in women (HR 1.16, 95% CI 1.04–1.31).

Conclusion(s): The incidence of morphometric VFs in Japan was 14.5/1,000 person-years. The significant risk factors for incident VFs were age, female sex, the presence of MD at baseline, the presence of VF at baseline, and advanced age of menarche.

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COP29

Opportunistic screening for osteoporosis using abdominal computed tomography scans in patients awaiting orthotopic liver transplantation identifies CT-attenuation as the major risk factor for vertebral fractures

Clément Nachef[a], Valérie Bousson[b], François Durand[c], Thomas Funck-Brentano[a]
[a]*Université de Paris, Rheumatology, Paris, France*
[b]*Université de Paris, Radiology, Paris, France*
[c]*Université de Paris, Hepatology, Clichy, France*

Background/Introduction: Patients with chronic liver disease are at high risk of osteoporosis as defined by low bone mineral density (BMD) or fragility fractures. The detection of patients at high risk of fracture is a medical need.

Purpose: Our objective was to identify risk factors for prevalent VF using opportunistic screening on CT scans.

Methods: We retrospectively analyzed CT-scans in a prospective cohort from an academic liver transplant unit. We included all consecutive patients aged 18 and over that were listed for liver transplantation between March 2004 and June 2018. Prevalent VF were identified on sagittal and coronal reconstructions. CT-attenuation (mean Hounsfield unit) was measured in the L1 vertebral body.

Results: 376 patients were included in the final cohort (75% males; mean age 55 ± 8 years; mean BMI 26.3 ± 4.6 kg/m²; 42% alcoholic cirrhosis, 40% viral hepatitis cirrhosis). 139 VF in 55 (14.6%) patients were identified. In univariate analyses, age was modestly associated with prevalent VF (P=0.034). Liver-specific factors were associated with higher prevalence of VF, such as alcoholic cirrhosis (P< 0.001) and cirrhosis severity assessed by higher Child grade (P=0.018), refractory ascites (P=0.001) and encephalopathy (P=0.023). Notably, patients with VF had dramatically lower L1 CT-attenuation values (86.3 ± 30.3 vs 137.6 ± 40.2 HU, P<0.001). Logistic regression models adjusted for age, sex, BMI, alcoholic cirrhosis and Child showed that low CT-attenuation was the only independent determinant of prevalent vertebral fracture (for each SD decrease, OR=7.00 95%CI [4.00 to 12.35]. L1 CT-attenuation below 100 HU was associated with greater risk of prevalent VF (OR=8.7 95%CI [4.5 to 16.6]).

Conclusion(s): In patients awaiting liver transplantation, low CT-attenuation of the L1 vertebra is an easy tool to identify patients at high risk of vertebral fractures.

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COP30

Bone analysis revealed high bone resorption in idiopathic osteoporosis in young adults

Bastien Leger, Agnes Ostertag, Thomas Funck-Brentano, Caroline Marty, Corinne Collet, Martine Cohen-Solal
Hopital Lariboisiere, Rheumatology, Paris, France

Background/Introduction: Idiopathic osteoporosis (IO) is uncommon disorder in young adults before the age <50-55 years. The pathogenesis is reported as an osteoblast dysfunction.

Purpose: The aim of the study was to describe the histomorphometric profile in IO.

Methods: From 2010 to 2019, histomorphometry analysis was performed on 18 bone biopsies of IO patients, without secondary causes. Biopsies of IO patients were compared to a group of 31 untreated postmenopausal osteoporotic women (U-OP) and to 16 controls without osteoporosis (Ct). Bone biopsies were analyzed by microcomputed tomography for the microarchitecture and histomorphometric parameters. Serum CTX levels were measured.

Results: Histomorphometry analysis revealed no difference in BV/TV (17.4±6.6 vs 17.6±5.2 vs 15.1±5.1%) between the 3 groups. In IO compared to U-OP and Ct, trabecular number was higher (Tb.N ; 1.3±0.391 vs 1.1±0.2.64 vs 0.953±0.313/mm, p<0.05), while Trabecular Thickness was lower (Tb.Th, 137.3±30.8 vs 153.7±30.8 vs 160.5±5.0 µm, p<0.05). The Osteoblast surfaces (OS/BS %, 2.4±2.5 vs 3±0.637 vs 2±1.7), Eroded surfaces (Er.S/BS, 1.1±0.836 vs 0.585 ±0.080 vs 0.588±0.350%, p<0.05) and Osteoclast number (N.Oc/mm3, 0.924±0.602 vs 0.199±0.070 vs 0.574±0.458, p<0.05) were higher in IO compared to U-OP and Ct. Labelling was present in 64.7% (11/17) in IO vs 80.6% (25/31) in U-OP. Cortical porosity, cortical thickness, and bone mineral density in both cortical and trabecular bone did not shown any statistical differences. CTX (ng/mL) levels were also higher (1125±678 vs 447.6±387.7).

Conclusion(s): Here, we show a large variation in the remodeling rate in IO patients. This included low bone formation and unexpected high bone resorption compared to U-OP and controls women despite older age. The persistent bone resorption in IO might be responsible to bone fragility in addition to reported reduction in bone formation. This study could have implications for the therapeutical management in young patients with osteoporosis.

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Concurrent Oral Presentations 3: Basic / Translational: Metabolism, Hormones and Growth Fractures

COP31

Glucose metabolism via the pentose phosphate pathway controls chondrocyte survival and proteostasis during bone development

Shauni Loopmans[a], Steve Stegen[a], Ingrid Stockmans[a], Karen Moermans[a], Peter Carmeliet[b], Geert Carmeliet[a]

[a]KU Leuven, Clinical and Experimental Endocrinology CHROMETA, Leuven, Belgium
[b]KU Leuven, Angiogenesis and Vascular Metabolism - VIB, Leuven, Belgium

Background/Introduction: Proliferation and matrix synthesis by growth plate chondrocytes are essential for proper bone development. To fulfill these anabolic functions in an avascular environment, chondrocytes need to be metabolically adapted to this nutrient-restricted microenvironment.

Purpose: Our single-cell transcriptomic profiling of neonatal growth plates shows that glucose metabolism via the pentose phosphate pathway (PPP) increases with chondrocyte maturation. Generally, the PPP is considered to regulate redox homeostasis and cell proliferation through the synthesis of NADPH and ribose-5-phosphate for nucleotide synthesis, but its role in chondrocytes is completely unknown.

Methods: To determine whether and how the PPP in chondrocytes regulates endochondral ossification, we deleted glucose-6-phosphate dehydrogenase (G6PDH), the rate-limiting enzyme of the PPP, in these cells using *Col2*-Cre mice (*G6pdh*$^{chon-}$).

Results: *G6pdh*$^{chon-}$ mice had shorter long bones at post-natal day (PD) 3 as evidenced by decreased tibia length (-20%; n=15; p<0.001). Histological analysis of the tibia at PD3 showed smaller growth plates (-18%; n=15; p<0.001) with disorganization of the columnar and hypertrophic zone. The observed hypocellularity (-25%; n=8; p<0.01) was not due to decreased proliferation, assessed by BrdU incorporation. Instead, we detected only in *G6pdh*$^{chon-}$ mice pronounced cell death in the central region of the growth plate, analyzed by TUNEL staining (n=11). This central region stained also positive for ATF4 in *G6pdh*$^{chon-}$ mice (n=8), indicative for an activated unfolded protein response and impaired protein homeostasis. *Ex vivo* evidence of proteostasis disturbance was provided by increased expression of genes involved in endoplasmic-reticulum associated protein degradation (ERAD), including *Edem1* (+20%; n=6-9; p<0.01) and *Man1a2* (+25%; n=6-9; p<0.05).

Conclusion(s): Taken together, the PPP controls chondrocyte survival and protein homeostasis, which are both critical for normal growth plate organization and bone lengthening.

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COP32

Ex vivo blockade of oxidative phosphorylation increases the regenerative potential of skeletal progenitors

Guillaume Tournaire[a], Shauni Loopmans[a], Steve Stegen[a], Sophie Torrekens[a], Nick van Gastel[b], Geert Carmeliet[a]
[a]KU Leuven, Chronic Diseases- Metabolism and Ageing, Leuven, Belgium
[b]Université Catholique de Louvain, de Duve Institute, Brussels, Belgium

Background/Introduction: Cell-based tissue engineering is a promising therapy to regenerate large bone defects, but it critically depends on the osteogenic potential of the implanted cells. The periosteum provides an interesting cell source of skeletal stem/progenitor cells (SSPCs), but these cells lose their SSPCs properties during *ex vivo* expansion.

Purpose: Since SSPCs reside in specific niches, typified by a poorly characterized nutritional micro-environment, we hypothesized that targeting cell metabolism of murine periosteal SSPCs during ex vivo expansion can preserve SSPCs resulting in improved bone healing.

Methods: We screened pharmacological inhibitors targeting several metabolic pathways and analyzed SSPCs properties.

Results: We found that inhibiting complex III of the electron transport chain (ETC) by antimycin A (AMA) preserved SSPCs, evidenced by increased capacity to form primary (+1.96±0.32 fold; p<0.0001; n=10) and secondary (+4.15±0.57 fold; p<0.003; n=4) colonies and by higher expression of SSPC markers (assessed by flow cytometry analysis; skeletal stem cells +2.38±1.12 fold; p=0.002; n=8; pre-bone, cartilage and stromal progenitors +1.41±0.48 fold; p=0.004; n=8; Chan et al., 2015). In addition, AMA-treated periosteal cells preserved their multipotency as evidenced by increased differentiation to chondrocytes (Alcian blue staining), osteoblasts (ALP/Alizarin red staining) and adipocytes (oil red O staining). AMA-treated cells formed more bone in an *in vivo* ectopic implantation assay (+3.73±1.16 fold; p=0.002; n=8) and this effect was linked to increased presence of SSPCs (+1.57±0.19 fold; p=0.001; n=6). Blocking ETC in tumor cells normally halts their proliferation, but this effect was not observed in periosteal cells because of metabolic adaptations. This normal proliferation allowed sufficient expansion, which is necessary for tissue engineering approaches.

Conclusion(s): Together our data show that blockade of mitochondrial complex III during *in vitro* expansion preserves SSPCs resulting in improved bone regeneration. Targeting cell metabolism is thus a promising approach to control cell renewal and fate *in vitro*, which is crucial for cell-based regenerative medicine.

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COP33

Phenotype of the first mouse model of Cole Carpenter Syndrome

Antonio Maurizi[a], Nadia Rucci[a], Antonella Stoppacciaro[b], Paolo Menè[b], Anna Teti[a]
[a]University of L'Aquila, Department of Biotechnological and Applied Clinical Sciences, L'Aquila, Italy
[b]University Sapienza, Department of Clinical and Molecular Medicine, Rome, Italy

Background/Introduction: The Cole Carpenter Syndrome (CCS) is a rare genetic disease displaying an autosomal dominant inheritance with a prevalence <1:1,000,000. It affects mainly the bone, which becomes brittle and fractures many times. Genetically, the CCS is caused by the heterozygous p.Y393C mutation in the *P4HB* gene encoding for the Prolyl 4-hydroxylase β subunit.

Purpose: We investigated the skeletal and non-skeletal phenotype of CCS mice in order to understand the disease pathogenesis.

Methods: A mouse model of CCS, carrying the aminoacidic substitution *tyr*393*cys* (*P4hb*^{Y393C}) mutation, was generated and subjected to in-depth phenotyping.

Results: The bone phenotype, assessed by μCT in 12-month-old male mice, showed a significant and severe reduction of the BV/TV (-48%;p=0.002) in *P4hb*^{Y393C} mice compared to WT littermates. Accordingly, the number of bone trabeculae (Tb.N) was lower (-51%;p=0.002) and their separation (Tb.Sp) was higher (+1.3fold; p=0.004) in *P4hb*^{Y393C} mice, while the trabecular thickness was unremarkable. Interestingly, the Indentation Distance and the Total Indentation Distance were dramatically increased in *P4hb*^{Y393C} femurs compared to WT (+1.73;+1.72fold, respectively,p<0.05), indicating a poor bone quality. Bone histomorphometry showed a significant reduction of osteoblast number and surface in *P4hb*^{Y393C} mice compared to WT (-36%;-39%; respectively, p<0.05) and gene expression analysis revealed lower *Runx2*, *Col1a1* and *Osteocalcin* mRNA levels (p<0.05) in *P4hb*^{Y393C} femurs. Moreover, extensive

tubular vacuolization along with intracellular protein accumulation, intraluminal protein casts and glomerular shrinkage were found in the kidney of *P4hb*^{Y393C} compared to WT, indicating renal injury/structural rearrangement. Finally, we found lower serum insulin levels in *P4hb*^{Y393C} mice compared to WT (-46%;p=0.02), suggesting pancreas involvement and impaired glucose metabolism.

Conclusion(s): A severe bone phenotype affects *P4hbY393C* mice, consistent with the skeletal features of the human disease. Organs beyond the bone, including kidney and pancreas, are also affected, suggesting a complex multiorgan involvement in the disease pathogenesis.

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COP34

Histone demethylase Kdm3a modulates bone formation in glucocorticoid-induced osteoporosis

Makoto Arai[a], Hiroyuki Hirakawa[b], Yoshihiro Matsumura[c], Juro Sakai[a,c]

[a]*Tohoku University, Division of Molecular Physiology and Metabolism-Graduate School of Medicine, Sendai- Miyagi, Japan*

[b]*Columbia University Irving Medical Center, Columbia Stem Cell Initiative, New York- NY, United States*

[c]*The University of Tokyo, Division of Metabolic Medicine- Research Center for Advanced Science and Technology, Tokyo, Japan*

Background/Introduction: Anti-osteoporosis medications have redressed the imbalance between bone resorption and formation. But options to restore bone formation are scarce. Also, bone remodeling is an unremitting process which we hypothesized to be modulated by epigenetic regulations.

Purpose: We aimed to elucidate epigenetic mechanisms of bone formation and their significance in pathological conditions.

Methods: We generated lysine demethylase 3a (Kdm3a)-null (KO) mice, and enzymatically inactive Kdm3a-H1122Y (HY) mice. At 12-week-old, bone volume and associated parameters were measured using micro computed tomography and histomorphometry. Comprehensive gene expression analysis was performed using Affymetrix DNA microarray. Single cell RNA-sequence was analyzed using 10X Genomics platform. All experiments were conducted with ethical approval.

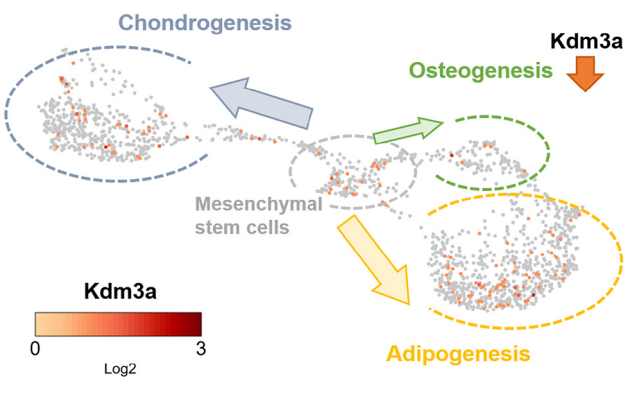

Figure. Kdm3a writes epigenetic codes of mesenchymal stem cells to deviate from osteogenic differentiation.

Results: Considering their long-term involvement in cellular memory, we focused on histone methylations and Kdm3a. Compared with wild type, KO mice showed higher bone volume (11.1 vs 15.6%; p<0.01) resulting from increased bone formation. Primary calvarial osteoblasts from KO mice showed enhanced ALP activities (745 vs 2842; p<0.01), while no differences were seen in osteoclasts. HY mice also had higher bone mass. Single cell RNA-sequence of bone tissue supported inhibitory effects of Kdm3a on osteogenesis (Figure). Through comprehensive search for Kdm3a target genes, we identified Pgk1 as the third largest reduction gene in Kdm3a knockdown. Pgk1 promotes apoptosis, and apoptosis in osteoblasts is pathognomonic in glucocorticoid-induced osteoporosis (GIO). Kdm3a knockdown in osteoblasts reduced Pgk1 expression, leading to attenuated apoptosis under dexamethasone (27.3 vs 18.4%; p<0.05).

Conclusion(s): We uncovered the significance of Kdm3a-mediated regulation of bone formation and its therapeutic possibility in GIO.

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COP35

Mast cells critically regulate bone repair and osteoclast activity under estrogen-deficient conditions

Verena Fischer[a], Deniz Ragipoglu[a], Johanna Diedrich[a], Anne Dudeck[b], Miriam Kalbitz[c], Florian Gebhard[c], Melanie Haffner-Luntzer[a], Anita Ignatius[a]

[a]*Institute of Orthopaedic Research and Biomechanics, University Medical Center Ulm, Ulm, Germany*

[b]*Institute for Molecular and Clinical Immunology, Otto-von-Guericke University Magdeburg, Magdeburg, Germany*

[c]*Department of Traumatology- Hand– Plastic– and Reconstructive Surgery, University Medical Center Ulm, Ulm, Germany*

Background/Introduction: Mast cells (MCs) are proposed to influence bone turnover, as more MCs were found in patients with osteoporosis. Supporting, we reported that MCs are involved in ovariectomy-(OVX)-induced bone loss and stimulate osteoclastogenesis during bone repair.

Purpose: Here, we studied if MCs are also involved in OVX-induced delayed bone repair using MC-deficient mice and investigated in a translational approach the effects of MC mediators on human osteoclasts in vitro.

Methods: Female Mcpt5-Cre R-DTA mice (Cre$^-$: MC-competent; Cre$^+$: MC-deficient; 12-weeks old) were OVX- or sham-operated and subjected to femur osteotomy. Bone repair was analyzed 6h and 21d after fracture by serum, µCT, biomechanical and histomorphometric analysis. Effects of conditioned medium of human HMC-1.2 cells incubated with/without estrogen on human primary osteoclast formation were examined in vitro, performing also ERalpha RNA interference and mediator release experiments.

Results: MC-competent OVX mice displayed an increased inflammatory response (IL-6, CXCL10) 6h after fracture compared to sham mice. Moreover, at d21 after fracture bone repair was impaired indicated by a significantly reduced bending stiffness (-46%), bone content (-24%) and size (-36%) of the callus. Osteoclast number and activity were increased in the callus of MC-competent OVX mice compared to sham mice (OVX vs sham: N.Oc/BPm (1/mm) 10.2 vs 7.5, p=0.0108; Oc.S/BS (%) 17.2 vs 11.8, p<0.0001). Interestingly, none of these differences between sham and OVX mice were observed in MC-deficient mice, indicating that MC-deficiency protects from negative effects of estrogen-deficiency. In vitro, conditioned MC-medium stimulated osteoclastogenesis, which was attenuated by estrogen (-48%) mediated via the ERalpha

receptor on MCs. Moreover, estrogen reduced the release of the osteoclastogenic factors CXCL10 (-42%) and Midkine (-67%) by MCs.

Conclusion(s): Concluding, our results imply an important role of MCs in bone repair under estrogen-deficient conditions by regulating osteoclast activity. Targeting MCs might be a therapeutic option to improve bone repair in postmenopausal osteoporosis.

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COP36

Extramedullary erythropoiesis is activated during hematoma phase of bone healing in mice

Drenka Trivanovic[a,b], Theresa Kreuzahler[a,b], Bianca Schlierf[a,b], Ana Rita Pereira[a,b], Maximilian Rudert[b,c], Marietta Herrmann[a,b]

[a]*University Hospital Wuerzburg, IZKF Group Tissue Regeneration in Musculoskeletal Diseases, Wuerzburg, Germany*

[b]*University of Wuerzburg, Bernhard-Heine-Center for Locomotion Research, Wuerzburg, Germany*

[c]*University of Wuerzburg, Department of Orthopedic Surgery- König-Ludwig-Haus, Wuerzburg, Germany*

Background/Introduction: The musculoskeletal and hematopoietic system interact closely during fracture healing. Although the importance of bone marrow (BM) hematopoietic progenitor recruitment for bone regeneration was indicated, there is no evidence on extramedullary hematopoiesis (EMH) during bone healing.

Purpose: As hematopoietic emergencies occur during inflammation, we anticipate that inflammatory hematoma phase of bone healing may induce EMH and present novel evidence on the participation of splenic EMH in bone healing in mice.

Methods: Plate-fixed segmental femoral defects were created in female mice (C57BL/6J, n=74) (ethical approval 55.2.2-2532-2-580-23) and peripheral blood (PB), spleen and BM were collected 1, 3, and 7 days afterwards. Non-operated (control) or sham-operated mice (no bone defect) were also analyzed. *Ex vivo* examinations included hematological analyses, flow cytometry, RT-PCR, clonogenic and ELISA assays. Values are presented as mean ± SD and significance was estimated by Mann-Whitney test.

Results: PB analysis showed significantly decreased erythrocyte numbers (p=0,023) and hemoglobin level (p=0,032) at day-3. Protein level of EPO in PB plasma increased during healing and reached the highest value at day-7 (112,83±26,67) when compared to control group (23,67±10,26 pg/mL; p=0,039). Overall hematopoietic clonogenic activity was increased in the spleen at day-3 (p=0,04) and day-7 (p=0,047), while it was unaltered in BM. Frequencies of splenic erythroid colony-forming units were increased at day-3 (0,0472±0,0101%) when compared to control group (0,00125±0,0008%; p=0,0127). Percentages of splenic immature erythroid cells $Ter119^{-}CD71^{high}$, $Ter119^{+}CD71^{high}$, $Ter119^{+}CD71^{med}$, were elevated at day-3, and this was associated with increased c-kit expression in these populations. *EpoR* (p=0,041) and *BMP4* (p=0,021) mRNA expression was increased in splenocytes isolated at day-3. Hematoma-derived medium from day-1 increased *BMPR-II* mRNA expression in BM stromal cells.

Conclusion(s): Results suggest that stimulated extramedullary erythropoiesis during hematoma phase is controlled by EPO and BMP4, which in turn might govern bone regeneration, achieving their effects on hematopoietic as well as non-hematopoietic progenitors in BM.

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Concurrent Oral Poster Presentations

Concurrent Oral Poster Presentations 1: Clinical / Public Health: Osteoporosis

P142

Fracture distribution in elderly women: A FRISBEE sub-study

Aude Mugisha[a], Jean-Jacques Body[b], Virginie Kinnard[c], Laura Iconaru[d], Felicia Baleanu[d], Alexia Charles[d], Murielle Surquin[a], Rafik Karmali[d], Pierre Bergmann[e], Florence Benoit[a]

[a]*CHU Brugmann, Internal Medicine, Bruxelles, Belgium*

[b]*CHU Brugmann, Internal Medicine- Endocrinology- Translational Medicine, Bruxelles, Belgium*

[c]*CHU Brugmann, Nuclear Medicine, Bruxelles, Belgium*

[d]*CHU Brugmann, Endocrinology, Bruxelles, Belgium*

[e]*CHU Brugmann, Nuclear Medicine-Translational Medicine, Bruxelles, Belgium*

Background/Introduction: Though the epidemiology of classical MOFs is well known, the distribution of other fracture sites has been less studied.

Purpose: We examined the incidence of fractures in a cohort of elderly Belgian postmenopausal women and their distribution among skeletal sites as a function of age.

Methods: 3560 postmenopausal women, aged 60- 85 years (mean ± SD, 70.1±6.4 years), included in a prospective study from 2007 to 2013 were surveyed yearly (FRISBEE). The number of validated incident fractures was recorded according to age and site.

Results: 1336 fractures were recorded after a mean follow-up of 9.1 years. 756 fractures (57%) were categorized as MOFs and 580 (43%) as non-MOFs. When classifying fractures as central versus peripheral, there were 813 (61%) and 523 (39%) fractures, respectively. The increase of fracture incidence with age differed between fracture sites. We could thus consider three groups with a mean increase/decade (compared with the 60-69 years age group) of less than 1.5, 1.5-2.0 and 2.0-3.0. The greatest increase per decade was observed for the group including hip, scapula, pelvis, ribs, clinical spine, long bones diaphysis and clavicle fractures. The lowest increase was observed for metatarsal, metacarpal, tarsal, ankle and knee fractures. As a result, the ratio of MOFs to non-MOFs increased significantly with age, being 1.69 [95% CI: 1.42-2.01] for the subgroup 80-89 years versus 1.10 [0.83–1.45]) (P=0.017) for the 60-69 years group. The proportion of central fractures was also significantly higher in the group 80-89 years (2.57 [2.13- 3.09]) versus 0.91 [0.69- 1.2] (P<0.001) for the 60-69 years group.

Conclusion(s): The increase of fracture incidence with age varies widely between fracture sites. The fact that the incidence of some peripheral fractures does not increase significantly with age (e.g. ankle) suggests that bone fragility does not play a major role in their occurrence.

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P085

Type 2 diabetes clusters indicate diabetes duration key in fracture risk

Samuel Ghatan, Fariba Ahmadizar, Ruolin Li, M. Carola Zillikens, Carolina Medina-Gomez, Fernando Rivadenira, Maryam Kavousi, Ling Oei

Erasmus MC, Internal Medicine, Rotterdam, Netherlands

Background/Introduction: Individuals with type 2 diabetes mellitus (T2D) are at an increased risk of developing fractures, despite higher mean BMI and BMD. Recently, clinically-relevant sub-groups of T2D have been characterised using biomarkers of glycemic metabolism.

Purpose: Characterise T2D sub-groups in a population-based setting and test for differences in fracture risk.

Methods: A total of 10019 Rotterdam Study participants were available with glycemic and (incident) fracture follow-up. Participants with T2D (n=1678) were partitioned in subgroups using K-means clustering based on: HOMA-B, HOMA-IR, age of diabetes onset, BMI and waist circumference measurements. Non-vertebral fracture risk was estimated across T2D subgroups using Cox proportional hazard models, adjusted for sex, age, BMI, collection cohort and prevalent T2DM.

Results: Four T2D clusters were defined each with relatively-unique clinical characteristics namely, 1) advanced age of onset; 2) decreased insulin sensitivity; 3) beta-cell disfunction; 4) Obesity/high BMI. Individuals with prevalent and incident T2D (independent of cluster) had lower risk of fracture than non-diabetics (see Forest plot). In contrast, individuals with prevalent T2DM (n=1152) had increased risk of non-vertebral fracture (HR: 2.1, 95%CI: 1.65-2.76), than individuals without T2DM.

Conclusion(s): Despite that partitioning the heterogeneity of T2DM in clinically-meaningful clusters opens the road to tailored prevention and care, our findings with prevalent T2DM indicate that disease duration (likely with inadequate glycemic control) is the main determinant of fracture risk. In line with this contention, the association between T2DM and fracture risk is not causal, as causality requires association with incident cases, as also confirmed by earlier Mendelian randomization studies. Future work, using genetically-determined disease definitions and biomarkers will help unveil clusters of individuals with T2DM at increased risk of fracture.

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P072

Bone microarchitecture in patients with long-standing type 1 diabetes

Lilian Sewing[a], Laura Potasso[a], Sandra Baumann[a], Denis Schenk[b], Furkan Gazozcu[c], Kurt Lippuner[c], Philippe Zysset[b], Christian Meier[a]
[a]*University Hospital Basel, Div. of Endocrinology- Diabetes and Metabolism, Basel, Switzerland*
[b]*University of Bern, ARTORG Center, Bern, Switzerland*
[c]*University Hospital Bern, Dept. of Osteoporosis, Bern, Switzerland*

Background/Introduction: Type 1 diabetes (T1DM) is associated with a high fracture risk, specifically at non-vertebral sites. The influence of glycemic control and microvascular disease on skeletal health in long-standing T1DM remains largely unknown.

Purpose: We aimed to assess areal (aBMD) and volumetric bone mineral density (vBMD), bone microarchitecture and bone turnover in patients with long-standing T1DM, defined as disease duration >25 years.

Methods: We recruited 59 patients with T1DM (disease duration, 37.7 ±9.0 yrs.; age, 59.9±9.9 yrs.; BMI 25.5±3.7 kg/m^2; 5-year median HbA1c 7.1% [IQR 6.82-7.40] and 77 non-diabetic controls. Dual-energy X-ray absorptiometry (DXA), high-resolution peripheral quantitative computed tomography (HRpQCT) at the ultradistal radius and tibia and biochemical markers of bone turnover were assessed. Group comparisons were performed after adjustment for age, sex and BMI.

Results: Patients with T1DM had lower aBMD at the hip (p<0.001), distal radius (p=0.01), lumbar spine (p=0.04) and femoral neck (p=0.05) as compared to controls. CTX as a marker of bone resorption was significantly lower in T1DM (p=0.005). At the distal radius there were no significant differences in vBMD and bone microarchitecture. In contrast, patients with T1DM exhibited lower cortical thickness (estimate, -0.14 [-0.24, -0.04], p=0.004), smaller cortical perimeter (estimate -4.45 [-8.69, -0.2], p=0.04) and lower cortical vBMD (-28.66 [-54.38, -2.94], p=0.03) at the distal tibia. Presence of diabetic complications, specifically polyneuropathy, had only limited effects on bone structural parameters measured by HRpQCT.

Conclusion(s): Our results confirm that long-standing T1DM is a state of low bone turnover with reduced aBMD, especially at cortical bone sites. In contrast to previous data with shorter disease duration, there were no major changes in bone volumetry and microarchitecture in patients with T1DM or T1DM with microangiopathy, except for a cortical deficit at the ultradistal tibia. This discrepancy might be due to the overall good glycemic control in our population.

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P134

3D reconstruction of the femur based on 2D DXA images: An independent and extended evaluation

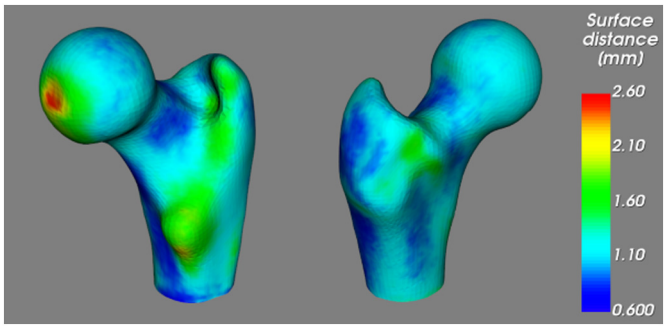
Alice Dudle[a], Michael Pretterklieber[b], Philippe Zysset[a]
[a]*ARTORG Center for Biomedical Engineering Research, University of Bern, Bern, Switzerland*
[b]*Center for Anatomy and Cell Biology, Medical University of Vienna, Vienna, Austria*

Background/Introduction: Osteoporosis diagnosis is currently based on aBMD measured with DXA scans. However, aBMD does not account for bone geometry or density distribution. QCT-based FE analysis estimates bone strength directly but implies higher costs and radiation doses. Instead, some studies have proposed to reconstruct 2D DXA scans into 3D images of the femur.

Purpose: We present the first independent and extended evaluation of the reconstruction method implemented in the software 3D-Shaper (Galgo Medical, Spain).

Methods: 83 human femora collected with the approval of the local ethics commission were scanned with CT and DXA scanners. DXA scans were reconstructed with 3D-Shaper and registered to the calibrated CT images.

Results: The volume of the reconstructed femur is underestimated compared to the CT image, with differences ranging from 3% in the shaft to 13% in the head. The average distance between 3D DXA and CT bone surfaces is illustrated in the figure below.



The 3D DXA and CT mean vBMD values show a correlation coefficient (R^2) of 0.92 but a systematic difference exceeding 30%, attributed to incoherent calibration of the devices. The voxel intensity correlation coefficient ranges from 0.21±0.11 in the head to 0.51±0.10 in the shaft, with the largest density differences located in the cortex.

Conclusion(s): The algorithm performs well in reconstructing the bone geometry but shows a systematic shift in the mean vBMD values and limitations in recovering the actual vBMD distributions.

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P135

Standardization of microstructural measurements of vertebral trabecular separation across different CT scanners for application in multicenter CT studies in osteoporosis

Jaime A. Peña, Reinhard Barkmann, Timo Damm, Claus-C. Glüer
Section Biomedical Imaging MOIN CC - Radiology and Neuroradiology, University Hospital Schleswig-Holstein UKSH, Kiel, Germany

Background/Introduction: In osteoporosis studies High Resolution Quantitative Computed Tomography (HR-QCT) allows trabecular separation (Tb.Sp) assessments in the vertebral spongiosa. However, different spatial resolution and reconstruction methods affect Tb.Sp. Standardization is thus required for accurate pooling of Tb.Sp data.

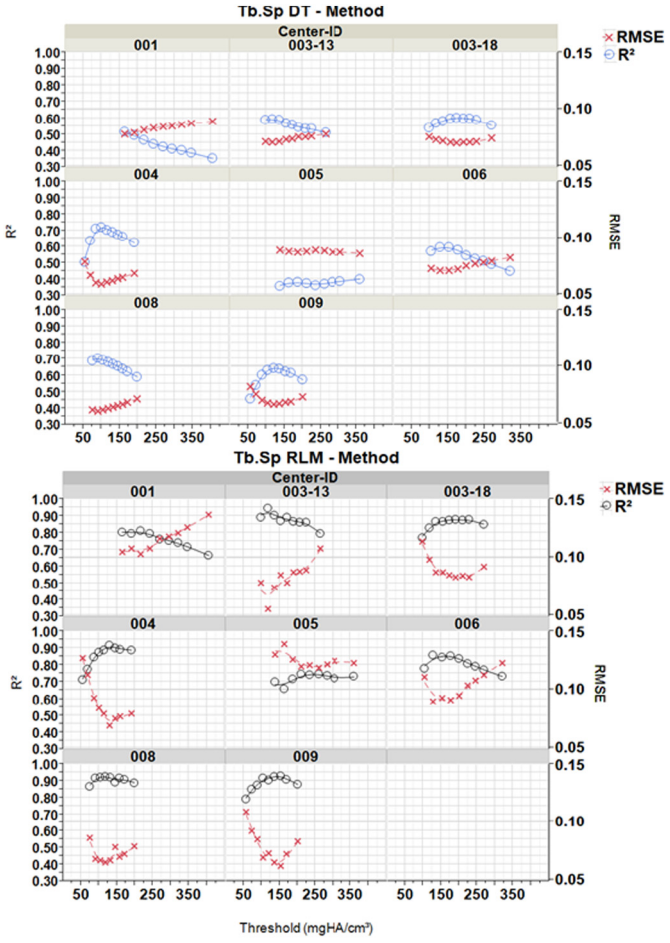
Purpose: Establish cross-calibration methodologies of microstructural measurements e.g. Tb.Sp for application in clinical multicenter studies.

Methods: Six human vertebral bodies were defatted, embedded in epoxy-resin and scanned inside an abdomen phantom for eight CT-scanners from different manufacturers. CT-volumes were density-calibrated and Tb.Sp for two methods was investigated. In-house implementations based on run-length (RLM) and the distance transform (DT) method. A gold-standard Tb.Sp (Tb.Sp_XCT) was obtained with XtremeCT (Scanco-Medical, Switzerland) for each method. For various thresholds RLM and DT estimations of Tb.Sp across all CT-centers were compared to Tb.Sp_XCT using linear regression to identify an optimal threshold (minRMSE, maxR^2) per-center and to compare the DT and RLM methods.

Results: Linear regression results for Tb.Sp_XCT versus Tb.Sp are presented in Fig.1. The overall best RMSE values per-center were between (0.059-0.086) for DT and (0.065-0.12) for RLM, respectively.

However, the R^2 values differed markedly with (0.402-0.719) for DT and (0.747-0.926) for RLM. Noticeably, only for some CT-centers an optimum threshold setting was observed.

Conclusion(s): The RLM method may show advantages over DT when structures like Tb.Bone are imaged with limited spatial-resolution and high image-noise. Further tests need to be performed to verify our observations and applications on patient data need to be done.



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P018

Development and external validation of nomograms for predicting one-year mortality and walking ability of Asian elderly femoral neck fracture patients after arthroplasty

Guangtao Fu
Guangdong Provincial People's Hospital, Orthopedics, Guangzhou, China

Background/Introduction: Femoral neck fractures are common in Asian elderly patients and related to high risk of mortality and walking ability impairment after hip arthroplasty. Simplified prognosis predicting models are strongly needed for preoperative clinical decision-making.

Purpose: The purpose of the present study was to create preoperative patient-specific factors-based nomograms for predicting mortality and walking ability of Asian elderly femoral neck fracture patients 1 year after arthroplasty.

Methods: Data of patients > 65 years who underwent primary unilateral hemiarthroplasty or total hip arthroplasty due to femoral neck fracture between January 1st, 2012 and June 30th, 2019 in our center were collected. Candidate variables included demographic data, comorbidities, and preoperative screening. Main outcomes included mortality and walking ability in the 1st postoperative year. Patients were randomly divided into derivation and validation groups. Nomograms were developed based on multiple logistic regressions of derivation group via R language. 1000 bootstraps were used for internal validation. Those models were further tested in the validation group and the entire data set.

Results: The final analysis was performed on 702 patients after screening. All-cause mortality one year after arthroplasty of the entire data set was 23.4%, while the independent walking rate was 74.4%. Age and preoperative walking ability showed the biggest impact on mortality and postoperative walking ability, respectively. The bias-corrected C index for predicting mortality in derivation group and the entire dataset were 0.717 and 0.712, while they were 0.754 and 0.733 for predicting walking ability impairment.

Conclusion(s): Our models enabled one-year mortality and walking ability predictions in Asian elderly femoral neck fracture patients with adequate predictive discrimination and calibration. Those models are helpful for surgeons in identifying high-risk patients and fulfilling rapid assessment before surgery.

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P116

Trabecular bone structure in advanced chronic kidney disease: Comparison of bone biopsy assessment using microCT and histomorphometry

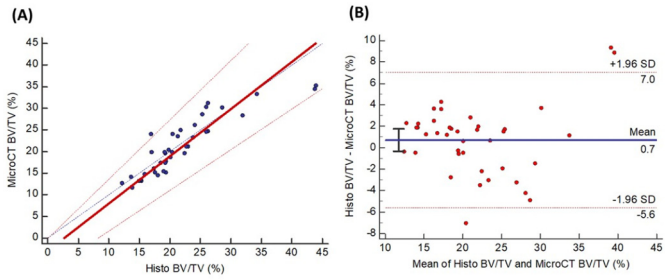
Syazrah Salam[a], Orla Gallagher[a], David Hughes[a], Arif Khwaja[b], Richard Eastell[a]
[a]*University of Sheffield, Department of Oncology and Metabolism, Sheffield, United Kingdom*
[b]*Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield Kidney Institute, Sheffield, United Kingdom*

Background/Introduction: Fracture risk is increased in advanced chronic kidney disease (CKD) patients due to bone abnormalities of renal osteodystrophy and osteoporosis. Bone biopsy is recommended before commencement of anti-resorptive therapy to assess bone turnover, mineralization and volume. MicroCT can assess bone volume and structure in bone biopsy samples quicker than quantitative histomorphometry.

Purpose: We aimed to assess if microCT and histomorphometry methods are interchangeable for trabecular bone volume and structure assessment of bone biopsy in advanced CKD.

Methods: 40 CKD stages 4-5D patients had trans-iliac bone biopsy using a 4mm diameter Jamshidi trephine. The bone biopsy core was scanned at 4.3μm resolution using the Skyscan MicroCT for assessment of trabecular bone volume/tissue volume (BV/TV) and structure. Then quantitative histomorphometry was performed using the Bioquant system. Normal mineralization was defined as osteoid thickness < 20μm. Methods comparison analysis was performed using the Passing-Bablok regression analysis and the Bland-Altman plot. The study was approved by the local ethics committee.

Results: All patients had normal mineralization status. The two methods showed good agreement for trabecular BV/TV assessment (Figure A). There was a mean of 0.7% (95% CI -0.33 to 1.74) underestimation of BV/TV by microCT (Figure B). The two methods also showed good agreement for measurements of trabecular thickness but not for trabecular number. There was a mean of 0.7% (95% CI -3.79 to 5.20) underestimation of trabecular thickness measurement by microCT.



(A) Passing-Bablock regression equation: MicroCT BV/TV = -3.13 + 1.10 (Histo BV/TV)
Intercept (95% CI) = -3.13 (-8.01 to -0.22) *Both methods differ by this constant amount*
Slope (95% CI) = 1.10 (0.95 to 1.37) *No proportional difference between the two methods*
Cusum test of linearity p=0.53. *No significant deviation from linearity*
(B) Bland-Altman plot: no bias

Conclusion(s): MicroCT and histomorphometry are interchangeable for trabecular bone volume and trabecular thickness assessments in advanced CKD patients with normal mineralization status.

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P147

Association between trabecular bone score and sleep patterns: Findings from a cohort study

Artemis Gkitakou[a,b], Fjorda Koromani[a], Katerina Trajanoska[a], Enisa Shevroja[d], M. Carola Zillikens[a], Ling Oei[a], Annemarie Luik[c], Didier Hans[d], Fernando Rivadeneira[a]
[a]*Erasmus University Medical Center, Internal Medicine, Rotterdam, Netherlands*
[b]*Lausanne University Hospital, Epidemiology, Lausanne, Switzerland*
[c]*Erasmus University Medical Center, Epidemiology, Rotterdam, Netherlands*
[d]*Lausanne University Hospital, Centre of Bone Diseases - Bone and Joint Department, Lausanne, Switzerland*

Background/Introduction: Sleep is a vital biological process involved in the regulation of a variety of metabolic and endocrine functions, including bone health. Previous studies have reported an association between sleep (duration and quality) and BMD in women, but findings remaining conflicting.

Purpose: Investigate the relationship between sleep quality, sleep duration and trabecular bone score (TBS) in a general population of elderly adults.

Methods: Study comprised participants from a population-based cohort of elderly individuals. During the visit at the research center, the participants underwent DXA assessment from which TBS from the lumbar spine was estimated. Sleep quality was assessed through the PSQI (Pittsburgh Sleep Quality Index), a self-rated 19- item questionnaire, which was filled out with the help of a research nurse. To test the association between all the sleep parameters and TBS we ran linear regression analyses adjusted for age, sex, cohort effect, BMI, education level, cholesterol levels, diabetes, alcohol use, depression symptoms, smoking status, history of cardiovascular disease. [FK1] We tested for interaction between sleep parameters and all covariates, and thereafter stratified the analysis for presence of depression symptoms.

Results: Among 5,534 individuals, 58.1% were women with a mean age of 66 years and sleep duration of 6.8 hours. In total 28.5% participants were classified as poor sleepers. A significant interaction was identified between depressive symptoms and sleep duration (P-value 0.03) and sleep quality (P=0.10). Participants defined as poor sleepers without depression symptoms had lower TBS compared to good sleepers (beta=-0.03, P-value=0.05) but not observed in those with depressive symptoms (beta=0.02, P-value=0.6). Longer duration of sleeping was associated with higher TBS (beta=.042, Pvalue=0.001) but not among individuals with depressive symptoms (beta=-0.03, Pvalue=0.40.

Conclusion(s): Poor sleep quality and duration are associated with low TBS only in individuals without depression symptoms.

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P151

The relationship between bone turnover and flu-like symptoms after zoledronate following denosumab discontinuation, the ZOLARMAB study

Anne Sophie Sølling, Torben Harsløf, Bente Langdahl
Aarhus University Hospital, Department of Endocrinology and Internal Medicine, Aarhus, Denmark

Background/Introduction: In ZOLARMAB, we investigated if treatment with zoledronate (ZOL) prevents BMD loss after denosumab (DMAB) discontinuation. 61% of our participants experienced flu-like symptoms after the first ZOL infusion.

Purpose: To investigate the relationship between bone turnover and other variables and the acute phase reactions (APR).

Methods: In ZOLARMAB, a randomized, open label, interventional study with 61 patients discontinuing DMAB after 4.6±1.6 years, we administrated ZOL 6 months (6M group) or 9 months (9M group) after the last DMAB or when bone turnover had increased (OBS group). We re-administrated ZOL if BMD decreased ≥5% or p-CTX increased ≥1.26 µg/L. Clinicaltrials NCT03087851.

Results: The majority of the patients from the 6M group experienced APR after ZOL, whereas it was well-balanced in the 9M and OBS groups (chi-sq, p=0.07) (table). All patients treated with ZOL based on the p-CTX criteria experienced APR. P-CTX, p-PINP, and p-BSAP at the time of ZOL treatment were similar between patients experiencing APR and those that did not, however, in the OBS group p-CTX was significantly higher among the patients experiencing APR (p<0.01). Only 14% of men, but 67% women experienced APR (p<0.01). Age, vitamin D status, duration of DMAB treatment, time since last DMAB, or previous bisphosphonate treatment were not associated with APR. Only 7% experienced APR after the second infusion.

	6-month group (n=20)		9-month group (n=20)		Observation group (n=21)		Total (n=61)	
Acute phase reaction	Yes	No	Yes	No	Yes	No	Yes	No
Number (%)	16 (80)	4 (20)	9 (45)	11 (55)	12 (57)	9 (43)	37 (61)	24 (39)
Mean p-CTX (µg)	0.20 ± 0.22	0.21 ± 0.13	0.90 ± 0.33	0.81 ± 0.30	1.2 ± 0.48	0.65 ± 0.20	0.67 ± 0.55	0.65 ± 0.32
Gender (female / male)	16 / 0	2 / 2	8 / 1	9 / 2	12 / 0	7 / 2	36 / 1	18 / 6
Months since last DMAB	6.0 ± 0	6.0 ± 0	8.8 ± 0.4	9.0 ± 0	9.6 ± 1.6	11.3 ± 1.3	7.8 ± 1.9	9.4 ± 2.0

Conclusion(s): High p-CTX levels and female gender might be associated with a high incidence of acute phase reaction after ZOL treatment following long-term DMAB.

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P183

Variants in the AMER1/WTX gene as a possible cause of idiopathic osteoporosis

Caroline Caetano[a], Manon Ricquebourg[a,b], Thomas Funck-Brentano[a,b], Philippe Orcel[a,b], Martine Cohen-Solal[a,b], Corinne Collet[a,c]
[a]*Inserm U1132, and Université de Paris, Paris, France*
[b]*Rheumatology, Hôpital Lariboisière- AP- HP, Paris, France*
[c]*UF de Biologie Moléculaire, Hôpital Lariboisière- AP- HP, Paris, France*

Background/Introduction: AMER1/WTX (APC Membrane Recruitment Protein 1) in the chromosome X encode a regulator protein of the canonical Wnt signaling pathway. AMER1 acts both as a positive and negative regulator of bone remodeling. A positive regulation is ensured by promoting LRP6 phosphorylation whilst a negative regulation acts as a scaffold protein for the degradation of beta-catenin complex, promoting stabilization of Axin at the cell membrane. Somatic and germline loss-of-function mutations of AMER1 are commonly associated with a human tumor suppressor in prevalent pediatric kidney cancers. Moreover, germline loss-of-function mutations of the *AMER1/WTX* predispose to osteopathia striata (OMIM 300373), a congenital X-linked dominant disease including cranial sclerosis generally lethal in men.

Purpose: From a cohort of 250 adults with idiopathic osteoporosis, we identified 7 patients (2.8%) with a missense variant with unknown signification in *AMER1/WTX* at heterozygous and at hemizygous level, associated with low BMD without typical imaging features of osteopathia striata. Two of the male patients presents exceptional variants (MAF <0.00005%) and another one has an undescribed mutation. Therefore, we conducted a preliminary functional study with the hemizygous variants to investigate a potential gain-of-function.

Methods: In silico software analysis (polyphen2, CADD and MutationTaster) revealed that 2 of the 3 variants are probably damaging. We generated an AMER1 mutated expression plasmid vector for each variant using the site directed mutagenesis method. The mutated plasmid vectors were transfected into HEK293 cell line, in order to assess the Wnt activity using TCF/LEF luciferase reporter system.

Results: No difference in Wnt activity was found in the presence of Wnt3a and Wnt5a recombinant proteins compared to the control, suggesting no involvement of the Wnt canonical pathway.

Conclusion(s): In conclusion, this first report of 7 patients with idiopathic osteoporosis carrying unexpected variants in the AMER1/WTX gene. Whether these variants are responsible for low BMD remain to be investigated.

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P105

Detraining effects on musculoskeletal parameters in early-postmenopausal osteopenic women – 3-month follow-up of the randomized controlled ACTLIFE-study

Wolfgang Kemmler[a], Simon von Stengel[a], Klaus Engelke[b]
[a]*Friedrich-Alexander-University FAU Erlangen-Nürnberg, Institute of Medical Physics, Erlangen, Germany*
[b]*Friedrich-Alexander-University FAU Erlangen-Nürnberg, Departement of Rheumatology and Immunology, Erlangen, Germany*

Background/Introduction: Periods of absence from supervised group exercise while maintaining physical activity might be a frequent pattern in adults' exercise habits. However, the corresponding detraining effects of short-moderate training breaks on musculoskeletal outcomes had been rarely evaluated.

Purpose: The aim of the present study was to determine detraining effects induced by the 3-month lock-down in Germany in Spring 2020 on musculoskeletal outcomes in early-post-menopausal, osteopenic women.

Methods: Due to the COVID-19 pandemic, we have to terminate the 18-month randomized controlled ACTLIFE exercise trial immediately after the 13-month follow-up assessment. While the high intensity aerobic and resistance group exercise sessions undertaken three times per week by the exercise group (EG: n=27) and the gentle exercise program performed once per week for the attention control group (CG: n=27) came to an abrupt stop due to the prohibition of group exercise, people were permitted to conduct individual outdoor activity during the 3-month lock-down period. Study endpoints were lean body mass (LBM), bone mineral density (BMD) at the lumbar spine (LS), maximum hip-/leg extension strength and power. The University Ethics Committee and the Federal Bureau of Radiation Protection approved the study.

Results: Detraining-induced reductions on LBM, hip/leg strength and power (but not BMD-LS) in the EG were significantly greater (p<.001 to p=.044) compared with the CG. Significant exercise effects, i.e. differences between EG and CG, present after 13 months of exercise, were lost after 3 months of detraining for LBM (p=.157) and BMD-LS (p=.065), but not for strength (p<.001) and power (p<.001). Of note, self-reported individual outdoor activities and (aerobic-type) exercise increased by »40% in both groups during the lock-down period.

Conclusion(s): Three months absence from a supervised group exercise protocol resulted in considerable detraining effects for musculoskeletal parameters. This indicate that exercise programs for adults should be in general continuous rather than intermittent with breaks ≥3 months.

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P140

Analysis of disparity in hip fracture rates between the five Danish healthcare regions 2014-2018

Bo Abrahamsen[a], Möller Sören[b]
[a]*Holbæk Hospital, Department of Medicine, Holbæk, Denmark*
[b]*Odense University Hospital and University of Southern Denmark, Open Patient Data Explorative Network, Odense, Denmark*

Background/Introduction: Despite the size of the country (pop 5.7 mio), there is a notable geographical variation in hip fracture rates. In March 2015, the Region of North Jutland introduced a joint plan for osteoporosis management between local authorities, hospitals, and primary practice. A small number of FLS services were established spread over the country.

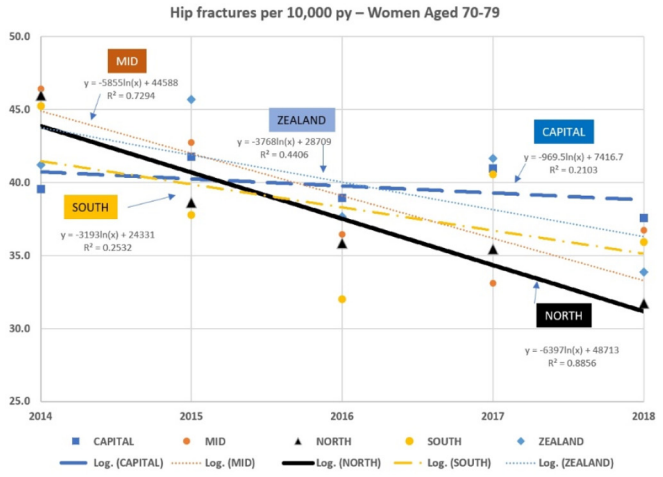
Purpose: The study aimed to investigate if rates decreased and if the variation in rates across the country changed.

Methods: Aggregate data on hip fracture surgery in the Danish populated aged 70 and over was stratified by age, sex and region and official resident demographics then used to calculate the population at risk for the denominator.

Results: In women, rates declined by 5 to 31% (fig 1) roughly following an exponential trend line (cf fig 2 for example with equations and intermediate years). In men aged 80 or over, rates declined by 2.6 to 24%. Rates became more homogenous (lower SD) across geographies in women but not in men.

Conclusion(s): Hip fracture rates decreased in both sexes and the variation in rates between the regions diminished in women but not in men. The largest reduction in hip fracture rates for women and for men aged 80 and over was seen in North Jutland, coinciding with upgraded osteoporosis services. Geographical differences in hip fracture risk parallelled the introduction of coordinated clinical management.

	Age 70-79 Hip fractures per 10,000 p.y.		Age 80+ Hip fractures per 10,000 p.y.	
Women	**2014**	**2018**	**2014**	**2018**
Capital reg.	39.6	37.6	155.1	140.5
Mid reg.	46.4	36.7	191.4	151.8
South reg.	45.2	35.9	179.1	169.0
Zealand reg.	41.2	33.9	200.8	157.0
North reg.	**46.0**	**31.7**	**188.9**	**139.3**
σ	*2.8*	*2.1*	*15.6*	*11.0*
Men				
Capital reg.	24.4	23.6	76.4	72.5
Mid reg.	28.6	30.3	99.9	89.5
South reg.	30.0	26.6	100.4	97.7
Zealand reg.	27.0	21.9	97.5	80.6
North reg.	**24.9**	**26.1**	**98.6**	**74.9**
σ	*2.1*	*2.8*	*9.1*	*9.4*



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Concurrent Oral Poster Presentations 1: Basic / Translational: Bone Diseases

P007

Involvement of Irisin in age-related osteoporosis: positive correlation with BMD in older adult patients and inhibitory effect on the senescent marker p21 in osteoblasts

Graziana Colaianni[a], Lorenzo Sanesi[b], Giuseppina Storlino[a], Roberta Zerlotin[a], Patrizia Pignataro[b], Cinzia Buccoliero[a], Manuela Dicarlo[b], Giacomina Brunetti[c], Mariella Errede[b], Maria Grano[a]

[a]*University of Bari, Department of Emergency and Organ Transplantation, Bari, Italy*

[b]*University of Bari, Department of Basic Medical Sciences- Neuroscience and Sense Organs, Bari, Italy*

[c]*University of Bari, Department of Bioscience- Biotechnology and Biopharmaceuticals, Bari, Italy*

Background/Introduction: We previously showed that treatment with the myokine Irisin ameliorates disuse-induced osteoporosis and muscular atrophy in mice. In humans, we and other authors showed a positive correlation between irisin and bone mineral density (BMD) in children and young adult athletes.

Purpose: Few studies have been conducted so far to investigate the links between circulating Irisin, its precursor, FNDC5, in skeletal muscle, and bone status in the same population of human subjects. Therefore, we evaluated possible correlations of Irisin serum levels with BMD and key parameters obtained from bone and muscle biopsies in a cohort of older adult patients. To address whether Irisin may be effective in delaying the cellular aging process, we also investigated its possible senolytic action on osteoblast cell cultures *in vitro*.

Methods: Sixty-two patients (age 68.71± 12.31) undergoing total hip or knee replacement were recruited and divided into osteopenic/osteoporotic patients and healthy subjects. Serum Irisin concentration, BMD, and in bone and muscle biopsies mRNAs of Osteocalcin (*Ocn*), Irisin precursor (*Fndc5*), and *p21* were measured.

Results: Irisin serum levels negatively correlated with age (r=-0.515; p=0.000018) and positively correlated with femoral (r=0.619; p=0.001) and vertebral (r=0.201; p=0.0001) BMD. Irisin was also positively associated with *Fndc5* mRNA in muscle biopsies (r=0.248; p=0.016), as well as with *Ocn* mRNA in bone biopsies (r=0.708; p=0.006). Of note, we found lower irisin levels (p=0.0011) in patients with osteopenia/osteoporosis (OP) compared to healthy controls. By analyzing the senescence marker *p21*, we found a significant increase in its expression in the bone biopsies of OP patients compared to controls. Additionally, *in vitro* data on murine osteoblasts showed that irisin downregulates *p21* mRNA levels (3-fold, p=0.03) compared to untreated cells.

Conclusion(s): Overall, these results indicate that, given the emerging role of irisin as an osteoanabolic agent, it could also represent a possible senolytic agent to delay age-related osteoporosis.

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P010

Repair of a critical size defect in osteoporotic mice

Franziska Strunz[a], Mark Siegrist[a], Nikola Saulacic[a], Marc Bohner[b], Willy Hofstetter[a]

[a]*University Bern, Department for BioMedical Research, Bern, Switzerland*

[b]*RMS Foundation, Bioceramics & Biocompatibility, Bettlach, Switzerland*

Background/Introduction: To prevent loss of bone mass and deterioration of microarchitecture in osteoporosis, bisophosphonates (BP) are the therapy of choice. Patients treated with BP, however, may still suffer from fractures and large bone defects. Biomaterials, such as CaP ceramics, may be used to fill critical-size defects, eventually in combination with osteogenic growth factors (bone morphogenetic protein-2; BMP2). L51P, an engineered BMP2 variant, which binds BMP antagonists, may cause an increase of the biological efficacy of BMP2.

Purpose: It is hypothesized, that prolonged BP therapy interferes with biomaterial turnover. To test this, the turnover of ßTCP implant ceramics was studied in a long bone critical-size defect in ovariectomized (*OVX*) mice treated with BP.

Methods: Eight weeks after *OVX*, treatment with BP commenced. Five weeks later, a femoral defect (3.5 mm) was generated and stabilized, using an internal osteosynthesis system. βTCP cylinders loaded with 0.25µg or 2.5µg BMP2, 2.5µg L51P, 0.25µg BMP2/2.5µg L51P and control implants were fitted into the defects. Femora were collected 6 and 12 weeks post-implantation.

Results: *OVX* led within eight weeks to a significant decrease in femoral total bone density in comparison to *sham* animals (p value 0.0001). In addition, analysis of uteri dry weight verified a shrinkage of uteri in *OVX* animals at the study endpoint (p value 0.0067). *OVX* mice under BP therapy, which received βTCP implants loaded with 0.25µg BMP2, 0.25µg BMP2/2.5µg L51P and 2.5µg BMP2, showed a strong induction of bone growth. In comparison, *OVX* animals without BP

medication showed formation of calcified tissue in the groups with 0.25μg BMP2/2.5μg L51P and 2.5μg BMP2 implants only.

Conclusion(s): The results indicate synergistic effects of BMP2 and L51P on bone healing. Moreover, BP caused a reduction in implant turnover. Therefore, efficiency of healing of biomaterial-filled bone defects might be impaired in patients treated with BP due to blocked implant removal.

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P159

Effect of hormone replacement therapy on bone formation quality and mineralization regulation mechanisms in early postmenopausal women

Sonja Gamsjaeger[a], Erik F. Eriksen[b], Eleftherios P. Paschalis[a]
[a]*Ludwig Boltzmann Institute of Osteology, at the Hanusch Hospital of OEGK and AUVA Trauma Centre Meidling- 1st Medical Department, Vienna, Austria*
[b]*Institute of Clinical Medicine- Oslo University Hospital, Department of Clinical Endocrinology- Morbid Obesity and Preventive Medicine, Oslo, Norway*

Background/Introduction: Post-menopausal osteoporosis is characterized by a negative imbalance between bone formation and bone resorption resulting in a net bone loss, increasing the risk of fracture. One of the earliest interventions to protect against this was hormonal replacement therapy (HRT).

Purpose: Bone strength depends on both the amount and quality of bone, the latter including compositional / material and structural properties. Bone compositional / material properties are greatly dependent on both patient-, and tissue-age.

Methods: Raman spectroscopy is an analytical tool ideally suited for the determination of bone compositional / material properties as a function of tissue age as it is capable of analyzing areas ~1 x 1 μm^2 in tetracycline labeled bone forming areas. Using such analysis of humeri from an ovariectomized primate animal model, we have previously shown that loss of estrogen results in alteration in the mineralization regulation mechanisms by osteoid organic matrix attributes at actively forming bone surfaces.

Results: In the present work we used Raman microspectroscopic analysis to analyze paired iliac crest biopsies obtained from 10 postmenopausal women at baseline and after 2 years treatment with HRT, to investigate the effects of this treatment on bone material / compositional properties at precisely defined micro-areas and tissue ages. Specifically, we analyzed osteoid, three tissue ages at forming cortical and trabecular surfaces (based on the presence of double fluorescent labels), and interstitial bone. The following parameters were measured: mineral / matrix ratio, mineral maturity / crystallinity, and tissue water, glycosaminoglycan, and pyridinoline content.

Conclusion(s): The results indicated significant correlations between osteoid proteoglycans, sulfated proteoglycans, pyridinoline, and earliest mineralized tissue mineral content, suggesting that in addition to changes in bone turnover rates, HRT affects the osteoid composition, as well as fibrillogenesis and mineralization regulation mechanisms.

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P104

Lrp5 mutant and crispant zebrafish faithfully model human osteoporosis, establishing the zebrafish as a platform for CRISPR-based functional screening of osteoporosis candidate genes

Jan Willem Bek[a], Chen Shochat[b], Adelbert De Clercq[a], Hanna De Saffel[a], Annekatrien Boel[a,c], Juriaan Metz[d], Frans Rodenburg[e,f,g], David Karasik[b,h], Andy Willaert[a], Paul Coucke[a]
[a]*Gent University, Biomolecular Medicine, Gent, Belgium*
[b]*Bar-Ilan University, The Musculoskeletal Genetics Laboratory, Safed, Israel*
[c]*Gent University, Department for reproductive Medicine, Gent, Belgium*
[d]*Radboud University, Department of Animal Ecology and Physiology, Nijmegen, Netherlands*
[e]*Leiden University, Institute of Biology, Leiden, Netherlands*
[f]*Tokyo Institute of Technology, School of Life Science and Technology, Tokyo, Japan*
[g]*Leiden University, Mathematical Institute, Leiden, Netherlands*
[h]*Harvard Medical School, Hinda and Arthur Marcus Institute for Aging Research, Boston, United States*

Background/Introduction: Genome-wide association studies (GWAS) have improved our understanding of the genetic architecture of common, complex diseases such as osteoporosis. Nevertheless, to attribute functional skeletal contributions of candidate genes to osteoporosis-related traits there is a need for efficient and cost-effective *in vivo* functional testing.

Purpose: This can be achieved through CRISPR-based reverse genetic screens, where phenotyping is traditionally performed in stable germline KO mutants. However, recently it was shown that first-generation (F0) mosaic mutant zebrafish (so-called crispants) recapitulate the phenotype of germline KOs.

Methods: To deliver proof-of-concept for functional validation of osteoporosis candidate genes through crispant screening, we compared a crispant to a stable KO zebrafish model for the *lrp5* gene. In humans, recessive loss-of-function mutations in *LRP5*, a co-receptor in the Wnt signaling pathway, cause Osteoporosis-pseudoglioma syndrome. In addition, several GWAS studies identified *LRP5* as a major risk locus for osteoporosis-related phenotypes.

Results: In this study, we showed that early stage *lrp5* KO larvae display decreased notochord mineralization ($P<0.0001$) and malformations of the head cartilage. Quantitative microCT and mass-spectrometry element analysis of the adult skeleton revealed decreased vertebral bone volume ($P<0.005$) and bone mineralization ($P<0.001$), hallmark features of osteoporosis. Furthermore, regenerating fin tissue displayed reduced Wnt signaling activity in *lrp5* KO adults. Additionally, *lrp5* crispants were generated by micro-injecting one-cell stage embryos with CRISPR RNP complexes containing Cas9 and a two-part gRNA (tracrRNA:crRNA duplex). Next-generation sequencing analysis of adult crispant tissue revealed a mean out-of-frame mutation rate of 76%, resulting in strongly reduced levels of Lrp5 protein. These crispants generally showed a milder, but nonetheless highly comparable skeletal phenotype and a similarly reduced Wnt pathway response compared to *lrp5* KO mutants.

Conclusion(s): In conclusion, we show through faithful modeling of LRP5-related primary osteoporosis, that crispant screening in zebrafish is a promising approach for rapid functional screening of osteoporosis candidate genes.

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P179

Early life stress does not affect bone mass in male mice but induces an osteopenic phenotype in female mice

Melanie Haffner-Luntzer[a], Dominik Langgartner[b], Giuila Mazzari[b], Julian Probst[b], Benjamin Krueger[a], Miriam Tschaffon[a], Anita Ignatius[a], Stefan O. Reber[b]
[a]*University of Ulm, Institute of Orthopaedic Research and Biomechanics, Ulm, Germany*
[b]*Laboratory for Molecular Psychosomatics- Department of Psychosomatic Medicine and Psychotherapy, University Ulm, Ulm, Germany*

Background/Introduction: Early life stress is a major risk factor for the development of mental diseases including depression, with women being three times more affected than men. The underlying mechanisms are not yet fully understood, but hyper-responsiveness of the HPA axis as well as activation of innate immunity might play an important role. Less is known about the influence of early life stress on bone metabolism.

Purpose: The aim of this study was to investigate how maternal separation, as a model for early life stress in rodents, influences bone parameters.

Methods: Female and male C57BL/6N mice were subjected to maternal separation (MS) for 3h per day on postnatal days 1-14. Weaning took place on d21. Control animals did not undergo MS, but were sham-handled (no MS animals). 6-7 weeks later, plasma was collected for cytokine/corticosterone analysis. Femurs were collected for μCT and histological analysis. n=10-12/group, Student's t-test.

Results: In male mice, MS did not have any significant effect on bone parameters. In female mice, MS animals displayed significantly reduced femur length, trabecular number, thickness and mineralization. Female MS mice displayed significantly increased numbers and activity of osteoclasts (NOc/BPm: noMS 8.74 ± 1.68 vs. MS 10.59 ± 1.41 1/mm; OcS/BS: noMS 14.83 ± 2.82 vs. MS 19.56 ± 2.47 %. p=0.015), whereas osteoblast parameters did not differ. No differences were found in plasma cytokine/corticosterone levels in male and female mice. However, female MS mice displayed significantly increased plasma levels of IL-6.

Conclusion(s): Male mice did not display alterations in bone parameters after early life stress, nor in inflammatory cytokine levels. In contrast, female mice displayed shorter long bones, an osteopenic bone phenotype and more osteoclasts. IL-6 is known to stimulate RANKL expression by osteoblasts which indirectly support osteoclast formation. The sex-related differences might be explained by estrogen and androgen-mediated effects on immune system activation under stress conditions.

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P038

Investigating the pathophysiological role of RANKL in mammary gland density and oncogenesis in osteoporotic TgRANKL mice

Anthi Kolokotroni[a,b], Evi Gkikopoulou[a,b], Vagelis Rinotas[a,b], Lydia Ntari[c], Danae Zareifi[d], Maritina Rouchota[e], Eirini Fragogeorgi[e], Ilias Lymperopoulos[f], Leonidas Alexopoulos[d], George Loudos[e], Maria Denis[c], Niki Karagianni[c], Eleni Douni[a,b]

[a]*Biomedical Sciences Research Center "Alexander Fleming", Institute for Bioinnovation, Athens, Greece*

[b]*Agricultural University of Athens, Department of Biotechnology, Athens, Greece*

[c]*Biomedcode Hellas SA, Research and Development, Athens, Greece*

[d]*National Technical University of Athens, Department of Mechanical Engineering, Athens, Greece*

[e]*Bioemission Technology Solutions, Bioemtech, Athens, Greece*

[f]*Iaso Hospital, 1rst Breast Clinic, Athens, Greece*

Background/Introduction: Receptor activator of nuclear factor-κB ligand (RANKL), is critically involved in the proliferation of mammary epithelial cells during pregnancy, as well as in mammary gland oncogenesis.

Purpose: In the present study, we investigated the effect of RANKL overexpression in mammary gland pathophysiology.

Methods: We performed a series of analyses in mammary glands of osteoporotic transgenic mice expressing human RANKL (TgRANKL), including expression of RANKL, structure and density of the epithelial ductal tree, tissue expression profile and susceptibility to carcinogenesis.

Results: Our results demonstrated expression of human RANKL at the luminal epithelial cells in ductal and alveolar structures that was further upregulated during pregnancy or upon synthetic progesterone (MPA) administration as quantified by qPCR (TgRANKL:1.33±0.66; TgRANKL+MPA:3.63±1.26, p<0.05). We also identified epithelial expansion in the mammary glands of TgRANKL mice compared to wild-type (WT) (Ductal branches volume/tissue volume, DBV/TV %, WT:4.363±1.808; TgRANKL:11.51±3.944, p<0.001) that was reversed by the RANKL inhibitor Denosumab (Dmab) as quantified by microCT (DBV/TV %, 7.237±1.046, p<0.05 between TgRANKL and TgRANKL +Dmab). Immunofluorescence analysis revealed increased epithelial cell proliferation shown by Cyclin D1 staining and expansion of mammary epithelial stem cells through flow cytometry. RNA sequencing and multi-plex protein analysis confirmed increased cell proliferation and identified deregulated gene expression and activation of signaling molecules associated with carcinogenesis. To investigate the incidence and progression of carcinogenesis in TgRANKL mice, we established an MPA-induced breast cancer model in WT and TgRANKL mice. Our results showed similar incidence in tumor development between WT and TgRANKL mice, which was significantly attenuated through pharmaceutical RANKL inhibition (Dmab) in TgRANKL mice (-84%, p<0.0001).

Conclusion(s): Collectively, our results demonstrated that overexpression of RANKL in the mammary glands promoted proliferation of mammary epithelial cells resulting in increased mammary density which is a risk factor for breast cancer, while preventive therapeutic inhibition of RANKL attenuated the incidence of mammary tumours.

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P041

Modifying of fibronectin accumulation pharmacologically diminishes bone metastasis growth by suppressing proliferation

Hiba Ghura, Stefan Hamelmann, Inaam Nakchbandi

Heidelberg University Hospital, Institute for Immunology and Serology, Heidelberg, Germany

Background/Introduction: Fibronectin is an extracellular matrix protein and higher fibronectin expression in breast and prostate cancer lesions shortens survival (von Au et al. Neoplasia). Fibronectin also suppresses the ability of myeloid cells to fight cancer (Rossnagl et al. PLoS Biology). Furthermore, matrix accumulation can be diminished pharmacologically (Altrock et al. J Hepatology).

Purpose: Investigation whether decreasing fibronectin accumulation in cancer will inhibit cancer growth.

Methods: Intratibial, Bioluminescence, Immunohistochemistry.

Results: Fibronectin was deleted (knockdown: Kd) in human breast cancer cells (MDA-MB-231) and cells injected intratibially in mice. Kd cells led to smaller tumors (Control (CT) 7±1 vs. Kd 1±0.3 ($x10^6$ RLU); p<0.0001). This was associated with a lower percentage of a-SMA- (a-smooth-muscle-actin)-expressing vessels (CT 60% vs. Kd 32%, p<0.05). Next, bone lesions of breast cancer were induced in mice and the peptide pUR4 that decreases fibronectin was administered for 10 days. This resulted in tumors half the size of control lesions (CT: 7±1 $x10^6$ RLU vs. pUR4: 4±1 $x10^6$ RLU, n=22/19, p<0.05), and was associated with a decrease in the percentage of a-SMA-expressing cells (CT 56 vs. pUR4 33%, p<0.05). Injecting a molecule that decreases collagen but not fibronectin (R1R2) did not affect cancer growth (p=ns), but still diminished the percentage of a-SMA-expressing cells (CT 67 vs. R1R2 43%, p<0.01). These data suggest that the maturity of blood vessels is not affected with modification of matrix. Instead, only a decrease in fibronectin suppresses cancer growth. Proliferation of cancer cells was therefore evaluated by staining ki67. This confirmed a decrease whenever

fibronectin was diminished; i.e., in Kd- and pUR4-treated tumors but not in R1R2-treated tumors (CT 19 vs. Kd 7; CT 25 vs. pUR4 10, CT 25 vs. R1R2 21%, $p<0.05$).

Conclusion(s): Taken together, these data show that decreasing fibronectin in tumors pharmacologically diminishes cancer growth by suppressing proliferation without any relevant effect on vessel development.

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P033

Targeted RNA-seq signature of breast cancer (BC) circulating tumor cells (CTCs) correlates with the onset of bone-only metastases

Stella D'oronzo[a], Domenica Lovero[a], Raffaele Palmirotta[b], Paola Cafforio[a], Janet Brown[c], Steven Wood[c], Camillo Porta[a], Franco Silvestris[a]

[a]*University of Bari Aldo Moro, Department of Biomedical Sciences and Human Oncology, Bari, Italy*

[b]*University of Bari Aldo Moro, Interdisciplinary of Medicine, Bari, Italy*

[c]*University of Sheffield, Oncology and Metabolism, Sheffield, United Kingdom*

Background/Introduction: Bone is the most common site of breast cancer (BC) metastases, but no biomarkers are currently available to predict skeletal dissemination.

Purpose: To identify a specific gene expression profile correlated with bone metastasis (BM) onset in CTCs from metastatic BC patients.

Methods: Following approval by local Ethics Committee and written informed consent, CTCs were isolated from 40 metastatic BC patients through immunomagnetic enrichment with autoMACS Separator® and DEPArray® sorting. A panel of 136 genes involved in the BM cascade was derived from literature data and standardized by targeted RNAseq on subclones of the MDA-MB231 BC cell line with different organotropism (P0: bone and viscera; P7: bone; LM: viscera). RNAseq was then performed on patients' CTCs, grouped in relation to the sites of radiologically-confirmed metastases, namely BM, extra-skeletal (ES) or both.

Results: The transcriptome heatmap of unsupervised hierarchical clustering of BC cell lines, based on normalized read counts, successfully separated the cell populations according to their organotropism.

The median number of isolated CTCs was 58 (range 18-95). Once the targeted RNAseq was performed on these cells, by considering an absolute fold change ≥ 2 and a false discovery rate threshold of 0.25, 31 DEGs were identified in BM versus ES CTCs (Figure 1).

Interestingly, according to Gene Ontology and KEGG pathway analyses, most DEGs were enriched in biological processes correlated with skeleton rearrangement.

Conclusion(s): CTCs are suitable biological sources for osteotropism investigation through targeted RNAseq. Wide prospective studies are needed to support our observations for clinical use.

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P031

Endoplasmic Reticulum (ER) stress: A role in bone metastatic breast cancer cellular dormancy

Katie Stoker, Nadia Rucci, Anna Teti, Antonio Maurizi

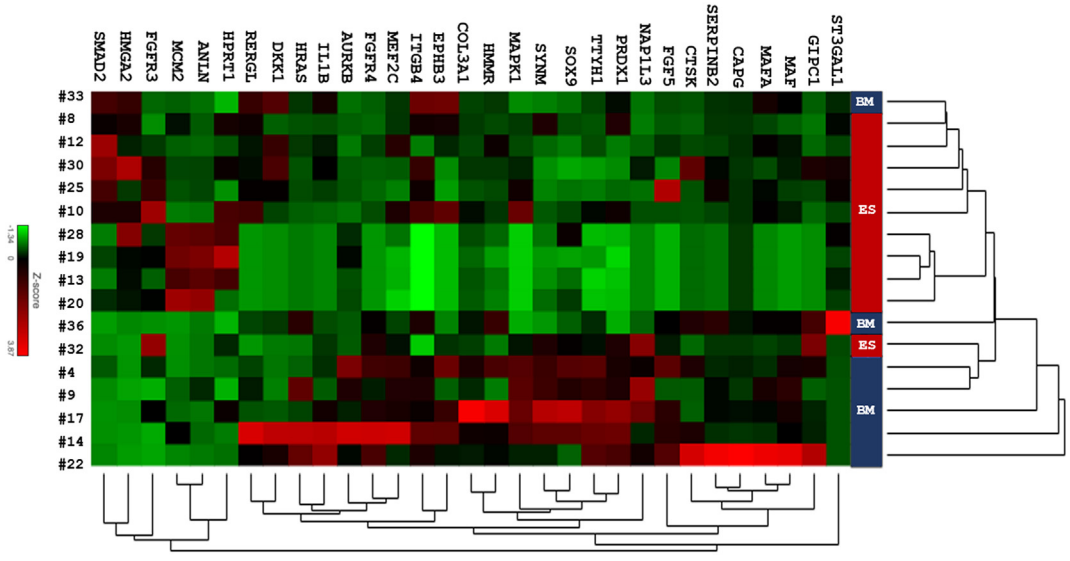
University of L'Aquila, Department of Biotechnological and Applied Clinical Sciences, L'Aquila, Italy

Background/Introduction: Tumour dormancy could be considered a mechanism of adaptation to stress conditions that gives a selective advantage to quiescent bone metastatic cells.

Purpose: We hypothesized a role for the ER stress in the induction and maintenance of dormancy.

Methods: We performed unbiased RNA dSeq in MDA-MB231 (MDA) cells expressing high level of Notch2 ($Notch2^{High}$), an established model of bone metastatic dormant cells.

Results: RNA dSeq analysis revealed differential expression of genes associated with ER stress in the $Notch2^{High}$ dormant MDA cells compared to $Notch2^{Low}$ MDA cells ($p<0.05$). Real time RT-PCR analysis confirmed a higher expression of the ER stress genes, *ATF3*, *DDIT3*, *EIF2α*, in $Notch2^{High}$ compared to $Notch2^{Low}$ MDA cells (+2.7,p=0.04; +1.8,p=0.05;+1.5fold,p=0.02, respectively). Network analysis showed that the identified ER stress markers interact with proteins encoded by the *DUSP1*, *IRF1* and *NGF* genes, found enriched in the $Notch2^{High}$ dormant cells by RNA dSeq ($p<0.05$) and confirmed by real time RT-PCR (+1.7,p=0.05;+1.6,p=0.05;+2.3fold,p=0.03 vs $Notch2^{Low}$, respectively, $p<0.05$), suggesting the presence of an ER stress molecular network in the dormant cells. Canonical ER stress markers and UPR target genes, including *BIP1*, *IRE1*, *PERK*, *ATF4*, *HERP*, *WARS*, *EDEM1* and *GADD34*, were significantly higher in the $Notch2^{High}$ MDA cells (average fold: +1.5 vs $Nocth2^{Low}$, $p<0.05$). However,



treatment of Notch2^{High} MDA cells with the ER stress inducer DL-Dithiothreitol (DTT), triggered no ER stress marker overexpression compared to Notch2^{Low} MDA cells. In contrast, some ER stress genes, including *ATF4*, *ATF3*, *DUSP1* and *NGF*, were significantly downregulated in Notch2^{High} MDA cells upon DTT treatment (-21%;-40%;-30%;-23%, respectively, $p<0.001$), indicating a lower UPR response that could protect Notch2^{High} MDA from the deleterious effects of the ER stress.

Conclusion(s): Overall, our results indicated the expression of an ER stress molecular network in Notch2High MDA cells that could represent a relevant pathway of dormancy and long-term resistance in the bone metastatic microenvironment.

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P126

Prediction of total knee arthroplasty risk using fractal analysis of radiographic trabecular bone texture: Data from the Osteoarthritis Initiative

Ahmad Almhdie-Imjabbar[a,b], Hechmi Toumi[a,b,c], Eric Lespessailles[a,b,c]
[a]*University of Orleans, EA 4708- I3MTO Laboratory, Orleans, France*
[b]*Hospital of Orleans, Translational Medicine Research Platform- PRIMMO, Orleans, France*
[c]*Hospital of Orleans, Department of Rheumatology, Orleans, France*

Background/Introduction: Knee replacement is considered as the ultimate and final outcomes of knee osteoarthritis (KOA) and thus it is of upmost importance to determine the most relevant factors that are associated with the occurrence of total knee arthroplasty (TKA).

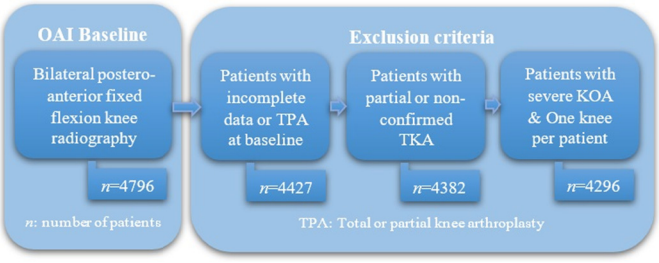
Purpose: Prediction of TKA risk in patients with KOA using fractal analysis of radiographic trabecular bone texture (TBT).

Methods: This study used data from the Osteoarthritis Initiative (OAI) cohort. Cases were defined as patients with TKA at least on one knee after 12 months, and controls were defined as patients who have never underwent a TKA prior to the 108-months follow-up control. Figure 1 summarize the dataset selection process.

The fractal parameters were computed using the Variogram (VAR) method.

Several prediction models based on TBT analysis were performed using logistic regression and compared to a recently proposed deep learning (DL)-based model (https://doi.org/10.1148/radiol.2020192091). The area under the receiver operating characteristic curve (AUC) curves were used to evaluate the global performance of considered models.

Results: 4296 knees (291 cases) were judged as eligible for this study. The proposed TBT-KL prediction model, based on the combination of TBT-based parameters and radiological Kellgren-Lawrence (KL) grades, achieved an AUC of 0.89, outperforming a reference prediction model based only on KL grades (AUC of 0.81). The proposed TBT-KL model also showed a better performance than the DL-based model (AUC=0.87).



Conclusion(s): The proposed TBT-KL model, involving TBT analysis of conventional knee radiographs, improved the capacity of predicting long term TKA risk in patients with KOA.

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P099

Associations between chondrocyte transiency, bone growth and osteoarthritis pathology in surgical and non-invasive loaded murine models

Hasmik Jasmine Samvelyan[a], Kamel Madi[b], Anna E. Törnqvist[c], Behzad Javaheri[d], Katherine Staines[a]
[a]*University of Brighton, School of Pharmacy and Biomolecular Sciences, Brighton, United Kingdom*
[b]*3Dmagination Ltd, Harwell Campus, Didcot, United Kingdom*
[c]*University of Gothenburg, Centre for Bone and Arthritis Research, Gothenburg, Sweden*
[d]*City University of London, Computer Science and Engineering, London, United Kingdom*

Background/Introduction: We have previously shown in a spontaneous murine model of osteoarthritis (OA); STR/ort mice, articular cartilage (AC) chondrocytes undergo transformation from inherently stable to transient phenotype revealing associations between growth abnormalities and OA.

Purpose: We hypothesised that altered growth dynamics underpin OA predisposition in surgical and non-invasive (loaded) models of OA.

Methods: Eight 8-week-old C57BL/6 male mice underwent DMM surgery to induce OA-like changes in right knee joints *in vivo*. Contralateral left knee joints had no intervention (controls). In 16-week-old C57BL/6 male mice ($n=6$), OA was induced using non-invasive mechanical loading of right knee joints with peak force of 11N. Non-loaded left knee joints were internal controls. Chondrocyte transiency in AC and growth plate (GP) of tibiae was examined by histology and immunohistochemistry. Tibial SCB parameters were measured using microCT and correlated to GP bridging. All procedures were approved by University of Edinburgh Ethics Committee.

Results: Higher expression of chondrocyte hypertrophy markers; Col10a1 and MMP13 were observed in tibial AC chondrocytes of DMM and loaded knees. In tibial GP, Col10a1 and MMP13 expressions were widely dispersed in enlarged zones of proliferative and hypertrophic chondrocytes. 3D quantification revealed enriched GP bridging and higher bridge densities in medial compared to lateral tibiae of DMM and loaded knee joints of the mice. GP dynamics were associated with higher SCB volume fraction in medial compared to lateral tibiae of DMM and loaded knee joints (DMM SCB BV/TV: 36.7±4.5% vs 29.4±3.1%, $p=0.03$; loaded: 47.1±1.6% vs 40.1±1.7%, $p=0.02$ respectively). and epiphyseal trabecular BV/TV in medial tibiae of loaded knee joints (73.4±2.8% vs 60.7±1.8%, $p=0.005$).

Conclusion(s): The results confirm associations between aberrant chondrocyte hypertrophy marker expression and OA pathology in surgical and loaded murine models of OA. Spatial variations in GP bridging formation reveal accelerated cartilage-bone transitions which may contribute to anatomical variation in vulnerability to OA development in these models.

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P102

Acetabular and femoral bone marrow in patients with hip osteoarthrosis: Cellular landscape of immune and stromal compartments

Drenka Trivanovic[a,b], Janek Hader[b], Maximilian Leucht[b], Theresa Kreuzahler[a,b], Bianca Schlierf[a,b], Konstantin Horas[b,c], Maximilian Rudert[b,c], Boris Holzapfel[b,c], Marietta Herrmann[a,b]
[a]*University Hospital Wuerzburg, IZKF Group Tissue Regeneration in*

Musculoskeletal Diseases, Wuerzburg, Germany
[b]*University of Wuerzburg, Bernhard-Heine-Center for Locomotion Research, Wuerzburg, Germany*
[c]*University of Wuerzburg, Department of Orthopaedic Surgery- König-Ludwig-Haus, Wuerzburg, Germany*

Background/Introduction: Osteoarthrosis (OA) of the hip leads to destruction of cartilage and bone tissue, requiring arthroplasty. Immune and stromal cells were found to be important in OA-affected synovial fluid, while cell compartments of bone marrow (BM) involved in OA are not revealed.

Purpose: We investigated BM populations of acetabulum and proximal femur obtained from patients at different OA stages.

Methods: BM reaming samples, 71 acetabulum (age 63,83±12,09 years) and 21 matched proximal femur (age 63±12,09 years), were obtained from patients undergoing total hip arthroplasty. Patients underwent radiographic imaging and were scored in accordance with the Kellgren and Lawrence classification. The study was approved by local ethical committee (No. 187/18). Cells were analyzed combining *ex vivo* and *in vitro* assays, using flow cytometry, RT-PCR and histochemical staining. Normal distribution of data was analyzed using the D'Agostino & Pearson test. Correlations were calculated using Spearman's rank correlation (r).

Results: The frequency of $CD45^+CD3^+CD4^+$ T-lymphocytes was higher in acetabular (3,67±0,98%) BM when compared to femur (2,34± 0,37%), and increased with age (r=0,448; p=0,008). Frequencies of examined B-lineage cells were significantly lower in acetabulum, where acetabular $CD45^+CD3^+CD4^+CD25^+$ T-lymphocytes (2,98±1,07%) positively correlated with $CD19^+$ B-lymphocytes (1,57±0,97%) frequency. The incidence of $CD45^-CD34^-CD13^+CD73^+$ stromal cells was higher in acetabulum (0,0019±0,00065%) than in femur (0,0005± 0,0001%) (p=0,056), showing certain positive correlation with presence of clonogenic cells. A higher proliferation rate in acetabular mesenchymal stromal cells (MSCs) was also associated with increased β-galactosidase activity and expression of genes involved in the regulation of cell cycle and survival (*p16*, *p53*, *HiF1α*, *Survivin*), and immunomodulatory activity (*Il-7*, *Il-6*, *Ido-1*, *Ptgs2*, *Tgf-β1*). Acetabular MSCs displayed higher osteogenic and chondrogenic, but weaker adipogenic potential than their femoral counterparts.

Conclusion(s): Our findings suggest that hip OA severity and associated risk factors may correlate with marrow cell frequency and functionality, indicating the possible skeletal site-dependent behavior of marrow cells in OA.

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Concurrent Oral Poster Presentations 2: Clinical / Public Health: Rare and Metabolic Bone Diseases

P163
Incidence and severity of ectopic ossifications in 25 adult patients with X-linked hypophosphatemia

Hajime Kato, Koga Minae, Ito Nobuaki
The University of Tokyo Hospital, Division of Nephrology and Endocrinology, Tokyo, Japan

Background/Introduction: Adults with X-linked hypophosphatemia (XLH) present ectopic ossifications.

Purpose: To describe the incidence and severity of ectopic ossifications in adults with XLH.

Methods: A total of 25 adults with XLH went thorough investigations, including spinal computed tomography scans, X-rays of hip/knee joints and Achilles tendons. The index of ossification of the anterior/posterior longitudinal ligament and yellow ligament (OA/OP/OY index) and the sum of OA/OP/OY index (OS index) were utilized to evaluate the severity of ossification of the spinal ligament (OSL). The Kellgren-Lawrence (KL) classification was adopted to evaluate the severity of the hip/knee osteophytes. Comparison of nonnormally distributed variables between groups was assessed by the Mann-Whitney U test. To certify the association between the severity of OSL / KL grade and other biomarkers, the Spearman rank correlation was chosen.

Results: The participants consisted of 13 male patients and 12 female patients from 21 families, with a median age of 43 (range, 18-72) years. Among them, 20 patients (80%) showed OSL. The median OA/OP/OY/OS indices were 2 (0-22), 0 (0-15), 6 (0-13) and 12 (0-41), respectively. Hip/knee osteophytes were reported in 24 (96%) and 17 cases (68%). The median KL grade was 3 in the hip joint and 2 in the knee joint, and 18 cases (72%) developed enthesopathy in the Achilles tendon. Positive correlations were identified between OS index and age (p=0.004, r=0.54) ,knee KL grade and age (p=0.01, r=0.47). OS index was also inversely correlated with bone alkaliphosphatase (p=0.04, r=-0.40). We did not find significant differences in biomarkers between patients with OSL and those without OSL.

Conclusion(s): This study revealed a high prevalence and severity of ectopic ossification among adult patients with XLH. In cases with severe OSL or noticeable osteophytes around the hip/knee joints, undiagnosed XLH should be considered as an underlying condition.

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P178
Burosumab treatment in a child with cutaneous skeletal hypophosphatemia syndrome: A case report

Manal Khadora[a], Zulf Mughal[b]
[a]*Latifa Women and Children Hospital, Pediatric Endocrinology, Dubai, United Arab Emirates*
[b]*Royal Manchester Children's Hospital, Pediatric Endocrinology, Manchester, United Kingdom*

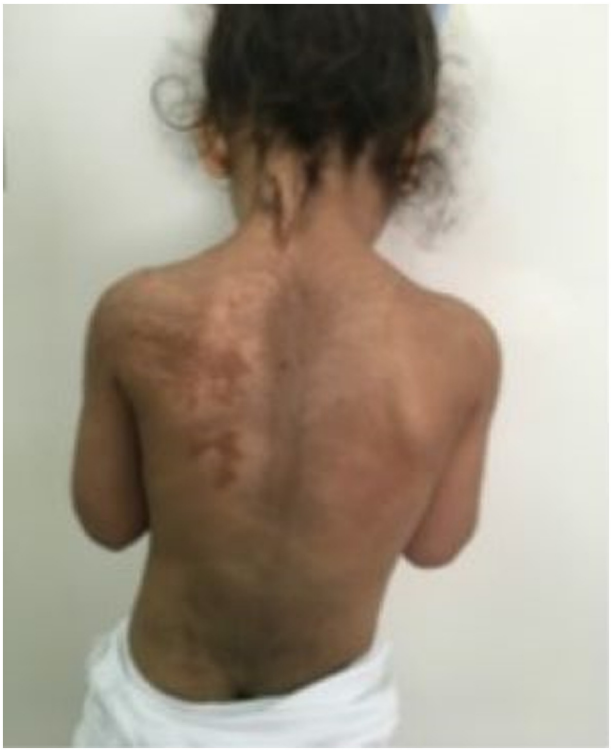
Background/Introduction: The cutaneous skeletal hypophosphatemia syndrome (CSHS) is a rare mosaic disorder caused by somatic gain-of-function *RAS* mutations. It is characterised by segmental epidermal nevi and fibroblast growth factor-23 (FGF23) mediated hypophosphatemic rickets. These patients also have dysplastic cortical skeletal lesions.

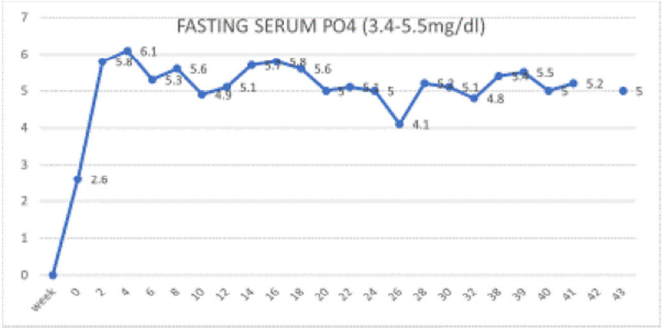
Purpose: We describe an Emirati child with CSHS, caused by somatic missense variant c.182A>G p.(Gin61Arg) (chr11:533874; hg19) in the *HRAS* gene.

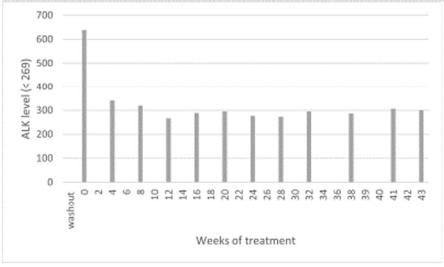
Methods: The diagnosis of CSHN with FGF23 mediated hypophosphataemic rickets was made in our patient due to increased urinary phosphate excretion and hypophosphatemia, in the face of normal serum PTH levels and inappropriately elevated plasma FGF23 levels. Her CSHN and dysplastic skeletal lesions failed to heal due to poor adherence to treatment with oral phosphate supplements and alfacalcidol.

Results: Treatment with burosumab, a fully human immunoglobulin G1 monoclonal antibody to FGF23 for 12 months, resulted in normalisation of her serum inorganic phosphate and alkaline phosphatase levels, healing of rickets, improvement in her symptoms of myopathy and quality of life.

Conclusion(s): Burosumab may have a role in the treatment of CSHS and dysplastic skeletal lesions.







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P188

Circulating sclerostin in adult patients with tumor-induced osteomalacia comparing with X-linked hypophosphatemia

Xiaolin Ni, Yan Jiang, Weibo Xia
Peking Union Medical College Hospital, Department of Endocrinology, Beijing, China

Background/Introduction: Sclerostin is an inhibitor of Wnt-β-catenin signaling to regulate bone mass. Tumor-induced osteomalacia (TIO) and X-linked hypophosphatemia (XLH) are the most common fibroblast growth factor 23 (FGF23)-dependent hypophosphatemic rickets/osteomalacia with abnormal bone mineral density (BMD). Up to date, circulating sclerostin level has been found to be elevated in XLH patients. However, it has not been explored in TIO patients which presented with low BMD.

Purpose: We aimed to evaluate serum sclerostin levels in TIO patients and the relationship between sclerostin levels with other clinical parameters.

Methods: Serum of TIO patients were collected for determination of sclerostin level by an ELISA kit and compared with that of age- and sex- matched controls and XLH patients. Relationship of circulating sclerostin level in TIO patients with BMD of lumbar spine, femoral neck and total hip and other clinical parameters were also analyzed.

Results: A total of 83 (46 men and 37 women) TIO patients aged 45.0(40.0, 54.0) [median (IQR)] years had lower circulating sclerostin [94.3(53.2, 115.9) pg/mL] than healthy control [104.8(74.5, 133.6) pg/mL] ($p=0.023$). Sclerostin levels were positively associated with age ($r=0.238$, $p=0.030$) in studied individuals and higher in male patients (103.3±46.6 pg/mL [mean±SD]) than in female patients (82.9±42.7 pg/mL) ($p=0.018$). In TIO patients, sclerostin level was positively associated with BMD of L1-4 ($r=0.264$, $p=0.018$), femoral neck ($r=0.264$, $p=0.018$) and total hip ($r=0.362$, $p=0.001$), as well as their corresponding Z-score of BMD. In addition to the similar finding of elevated circulating sclerostin level in XLH with previous studies, we firstly compared sclerostin levels in 14 XLH patients and age- and sex-matched TIO patients and found different levels (133.99±81.62 vs 61.87±29.45 pg/mL, $p=0.007$).

Conclusion(s): Our finding indicated that changed sclerostin levels in TIO and XLH patients might be compensatory consequence of altered BMD, providing evidence for potential application of antibody of sclerostin in these two diseases.

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P192

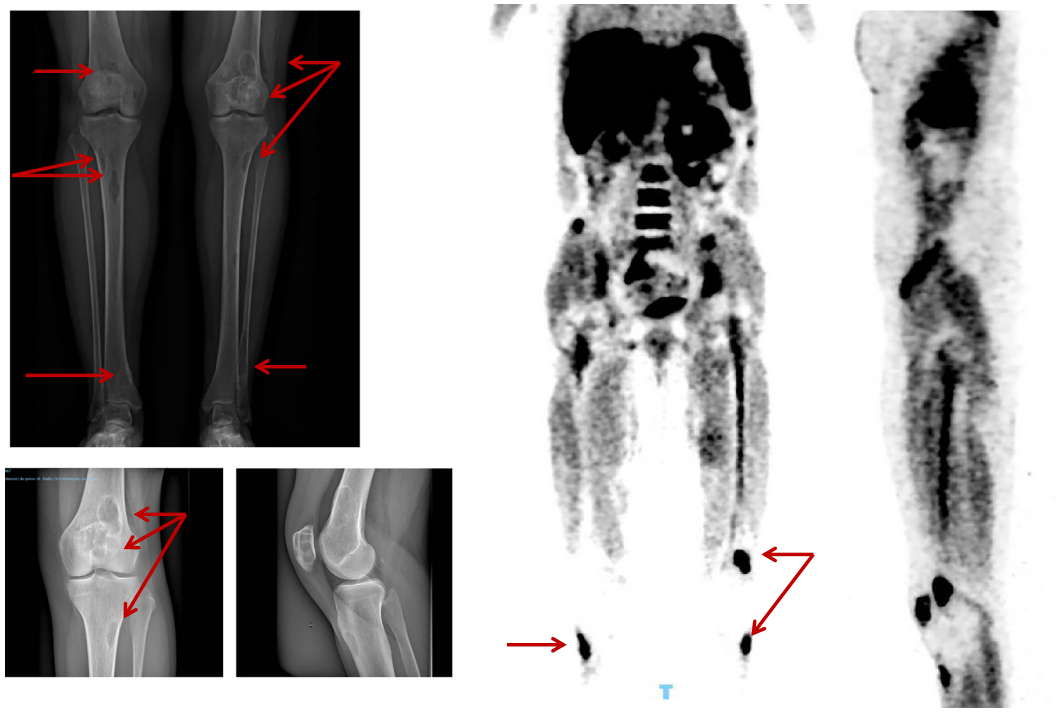
Multiple brown tumors: A severe bone complication of pseudohypoparathyroidism after 15 years of loss of follow-up

Sara Gonnelli[a], Karine Briot[a], Christian Roux[a], Stephanie Teboul[b], Catherine Cormier[a], Eugenie Koumakis[a]
[a]*Cochin Hospital, Rheumatology, Paris, France*
[b]*Mondor Hospital, Rheumatology, Paris, France*

Background/Introduction: Pseudohypoparathyroidism (PHP) is a metabolic disorder characterized by biochemical features of hypoparathyroidism (i.e., hypocalcemia and hyperphosphatemia) but with increased PTH due to resistance of target organs to PTH.

Methods: A 33 year-old woman was referred for the management of multiple bone lytic lesions. She had been complaining of knee pain since 2017. She had a history of asymptomatic PHP since her childhood, treated with alfacalcidol until 18 years-old, when she stopped because of the lack of symptoms. Radiographies and CT-scan revealed multiple lytic lesions of the metaphyseal regions of the distal left femur, proximal and distal tibias, and the left patella, which took up [99mTc] disphosphonate on bone scintigraphy. Bone biopsy of the femur revealed a giant cell-rich lesion without malignancy, indicating a brown tumor.

Results: Serum calcium was 83.2 mg/L,ionized calcium was 1.06 mmol/L (normal > 1.17), phosphate 1.16 mmol/L, alkaline phosphatase 232 UI/L), and PTH lwas 1386.8 pg/ml (12-88). A calcium load test was performed to assess parathyroid response: a large reduction in PTH from 1386.8 pg/ml to 349.1 pg/ml was observed leading us to rule out



Radiographies revealed multiple lytic lesions of the metaphyseal regions of the distal left femur, proximal and distal tibias, left patella, and left fibula, which took up 18F-fluorocholine

tertiary hyperparathyroidism and to consider the brown tumors as the result of bone responsiveness to PTH in this untreated patient.

Fluorocholine TEP imaging revealed multiple hypermetabolism consistent with radiographic lytic lesions of femur and tibias (Figure 1). Alfacalcidiol and calcium were reintroduced.

Conclusion(s): Resistance to PTH was initially postulated for bone as well as the kidney in PHP. However, radiological and histological features characteristic of osteitis fibrosa cystica have been described, this condition also termed hypohyperparathyroidism. To our knowledge this is the first report of multiple brown tumors imaged by fluorocholine in the course of PHP. This case emphasizes the importance of maintaining calcium and vitamin D supplementation in adult patients, to achieve PTH suppression and avoid hyperparathyroid bone disease.

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P194

The European Registry for Rare Bone and Mineral Conditions (EuRR-Bone): First year experience of the use of an e-reporting tool

Ana Luisa Priego Zurita[a], Agnès Linglart[b], Inês Alves[c], Renata Simona Auriemma[d], Manila Boarini[e], Jillian Bryce[f], Luisa DeSanctis[g], Diana-Alexandra Ertl[h], Hoong-Wei Gan[i], Corinna Grasemann[j], Wolfgang Högler[k], M. Kassim Javaid[l], Klaus Mohnike[m], Marina Mordenti[e], Adalbert Raimann[h], Luca Sangiorgi[e], Marco Roos[n], Rebecca Skarberg[o], Ondrej Soucek[p], Judit Toke[q], Erica van den Akker[r], Almudena Vicente[s], Ahmed S. Faisal[f], Natasha Appelman-Dijkstra[a]

[a]*Leiden University Medical Center, Endocrinology, Leiden, Netherlands*
[b]*Assistance Publique - Hopitaux de Paris, Department of Pediatric Endocrinology and Diabetology for Children, Paris, France*
[c]*ANDO Portugal, ERN Bond ePAG, Evora, Portugal*
[d]*Azienda Ospedaliera Universitaria "Federico II", Endocrinology, Napoli, Italy*
[e]*IRCCS Istituto Ortopedico Rizzoli, Department of Medical Genetics and Rare Orthopedic Diseases and CLIBI Laboratory, Bologna, Italy*
[f]*University of Glasgow, Developmental Endocrinology Research Group, Glasgow, United Kingdom*
[g]*Azienda Ospedaliero Universitaria Città della Salute e della Scienza di Torino, Department of Public Health and Pediatrics, Torino, Italy*
[h]*Medizinische Universität Wien, University Clinic for Pediatrics and Adolescent Medicine, Wien, Austria*
[i]*Great Ormond Street Hospital, Pediatric Endocrinology & Diabetes, London, United Kingdom*
[j]*Ruhr-University Bochum, Department of Pediatrics- St. Josef-Hospital, Bochum, Germany*
[k]*Johannes Kepler University Linz and Kepler University Hospital, Department of Pediatrics and Adolescent Medicine, Linz, Austria*
[l]*University of Oxford, Nuffield Department of Orthopaedics- Rheumatology & Musculoskeletal Sciences, Oxford, United Kingdom*
[m]*Otto-von-Guericke-Universitaet Magdeburg, Department of Pediatrics, Magdeburg, Germany*
[n]*Leiden University Medical Center, Human Genetics, Leiden, Netherlands*
[o]*OIFE, ERN Bond ePAG, Oslo, Norway*
[p]*Fakultni Nemocnice v Motole, Department of Pediatrics, Prague, Czech Republic*
[q]*Semmelweis Egyetem, Department of Internal Medicine, Budapest, Hungary*
[r]*Erasmus University Medical Center, Division of Pediatric Endocrinology- Department of Pediatrics- Sophia Children's Hospital, Rotterdam, Netherlands*
[s]*Hospital Virgen de la Salud, Department of Endocrinology and Nutrition, Toledo, Spain*

Background/Introduction: The European Registry for Rare Bone and Mineral Conditions (EuRR-Bone) was founded in April 2020 and is the affiliated registry of the European Reference Network for Rare Bone Diseases (ERN BOND).

Purpose: As bone and mineral conditions are seen by a variety of specialists there is a close connection with the European Registries for Rare Endocrine Conditions (EuRRECa) and its affiliated ERN Rare Endocrine Diseases (Endo-ERN). EuRR-Bone is open not only to

centres within these ERNs but also to others and consists of a core registry for all cases and an e-reporting registry for new cases.

Methods: The latter is accomplished via an electronic reporting platform, e-REC (e-Reporting of Rare Conditions), a light touch approach that does not collect personally identifiable information. Unique IDs for reported cases are generated instantaneously and emailed to users to be stored locally at reporting centres. Data are available to all collaborators following approval by the joint Data Access Committee of EuRRECa and EuRR-Bone.

Results: Until July 2020 , 12 centres from 9 different countries joined, of which 5 are ERN BOND members and 10 are Endo-ERN members. A total of 23 adults and 20 children were newly diagnosed with a Bone and Mineral condition. Amongst adults, the most frequently reported conditions were fibrous dysplasia and PTH independent hypercalcemia, while in children pseudohypoparathyroidism and osteogenesis imperfecta were the most reported. Since April 2020, the e-REC platform is also being used to capture the occurrence of a new COVID19 infection in a patient with an existing bone or mineral condition.

Conclusion(s): e-REC is a promising tool enabling clinical networks to objectively map conditions and related activity, providing a better understanding of the occurrence of the rare bone and mineral conditions.

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P086

A novel IFITM5 variant associates with phenotype of osteoporosis with calvarial doughnut lesions

Riikka Mäkitie[a,b], Minna Pekkinen[a,b], Naoya Morisada[c], Daisuke Kobayashi[d], Yoshiro Yonezawa[e], Gen Nishimura[f], Shiro Ikegawa[e], Outi Mäkitie[a,b]

[a]*Research Program for Clinical and Molecular Metabolism, Faculty of Medicine- University of Helsinki, Helsinki, Finland*

[b]*Folkhälsan, Institute of Genetics, Helsinki, Finland*

[c]*Hyogo Prefectural Kobe Children's Hospital, Department of Clinical Genetics, Kobe, Japan*

[d]*Hyogo Prefectural Kobe Children's Hospital, Department of Orthopaedic Surgery, Kobe, Japan*

[e]*RIKEN Center for Integrative Medical Sciences, Laboratory for Bone and Joint Diseases, Yokohama, Japan*

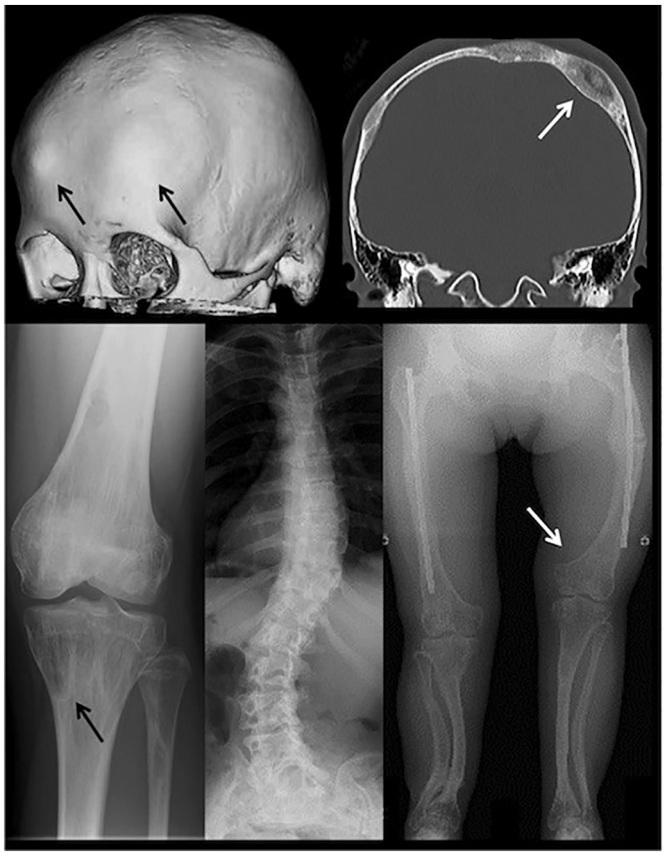
[f]*Saitama Medical University Hospital, Center for Intractable Disease, Saitama, Japan*

Background/Introduction: Mutations in *SGMS2*, encoding sphingomyelin synthetase 2, were recently discovered to underlie "Osteoporosis with calvarial doughnut lesions (OP-CDL)" disorder (OMIM #126550), characterized by moderate to severe skeletal fragility and prevalent fractures. As a peculiar feature, affected patients present with variable cranial hyperostotic lesions.

Purpose: This study expands the genetic causes of OP-CDL and cranial hyperostosis.

Methods: We identified a two-generation Japanese family with the skeletal phenotype of OP-CDL but with normal screening of *SGMS2*. Hence, we proceeded with whole-exome sequencing (WES) to look for a novel genetic cause. Genetic findings were complemented with detailed clinical evaluations and revisions of previously obtained skeletal and other clinical data.

Results: The affected patients, presently 18- and 47-year-old females, have severe, childhood-onset skeletal fragility with multiple long-bone fractures, scoliosis and skeletal deformities. Radiographs portrayed low and irregular mineralization with sclerotic striations, and bowing and metaphyseal overmodeling of tubular bones. Additionally, both exhibit multiple doughnut-shaped, sclerotic calvarial bumps (Figure 1). Through WES, we identified the genetic cause as a novel missense mutation c.143A>G (p.N48S) in the BRIL-encoding *IFITM5*-gene. Neither patient portrayed any of the classical features typically associated with *IFITM5*-related OI type V.



Conclusion(s): Our findings broaden the genetic spectrum of OP-CDL, suggest diversity in phenotypic characteristics stemming from *IFITM5* mutations and indicate a key role for BRIL in cranial skeletogenesis. Patients with OP-CDL-like phenotypes should be assessed for *IFITM5* variants. Further functional analyses evaluating potential molecular communication between BRIL and sphingomyelin metabolism are warranted.

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P190

Lethal osteogenesis imperfecta type XX caused by compound heterozygous frameshift mutations in MESD

Julian Stürznickel[a], Katharina Jaehn-Rickert[a], Jozef Zustin[a], Maximilian M. Delsmann[a], Helga Rehder[b], Anna Floriane Hennig[c], Katharina Schoner[d], Alfons Kreczy[e], Thorsten Schinke[a], Michael Amling[a], Uwe Kornak[c]

[a]*University Medical Center Hamburg-Eppendorf, Department of Osteology and Biomechanics, Hamburg, Germany*

[b]*Medical University of Vienna, Institute of Medical Genetics, Vienna, Austria*

[c]*University Medical Center Göttingen, Institute of Human Genetics, Göttingen, Germany*

[d]*Philipps-University Marburg, Institute of Pathology, Marburg, Germany*

[e]*REGIOMED Klinikum Coburg, Department of Pathology, Coburg, Germany*

Background/Introduction: Osteogenesis imperfecta (OI) comprises a phenotypically and genetically heterogenic bone disorder, primarily characterized by low bone mineral density and an increased risk of fractures.

Purpose: In addition to several known OI-associated genes, autosomal-recessive mesoderm development gene (*MESD*) mutations were previously described to cause autosomal-recessive OI XX.

Methods: We conducted whole-exome sequencing in three stillbirths of one family due to suspected hereditary disorder. Moreover, fetal autopsy, bone histology, and quantitative backscattered electron imaging (qBEI) were performed and compared to an age-matched control with regular skeletal phenotype.

Results: Compound heterozygous mutations in *MESD* exon 2 and exon 3 were detected in all three affected individuals. Their phenotype was characterized by multiple intrauterine fractures and severe skeletal deformity, leading to the diagnosis of OI XX. The *MESD* specimen showed an inferior bone development and, interestingly, a reduced osteocyte morphology and lacunocanalicular connectivity. Moreover, qBEI analysis revealed an altered and more heterogeneous matrix mineralization in individuals with *MESD* mutations compared to the control.

Conclusion(s): The severe phenotype is likely explained by the *MESD* mutation in exon 2, located within the chaperone domain, leading to complete loss-of-function and highlighting the relevance of *MESD* for early skeletal development.

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P164

The French Multicentre Elevated Bone Mass Study: Prevalence and causes

Julien Paccou[a], Rose-Marie Javier[b], Isabelle Henry-Desailly[c], Camille Ternynck[d], Aurore Nottez[a], Isabelle Legroux-Gérot[a], François Robin[e], Patrice Fardellone[c], Eric Lespessailles[f], Pascal Guggenbuhl[e], Christian Roux[g], Sami Kolta[g], Bernard Cortet[a]
[a]*Lille University Hospital, Rheumatology, Lille, France*
[b]*Strasbourg University Hospital, Rheumatology Unit, Strasbourg, France*
[c]*Picardie – Jules Verne University- Amiens Teaching Hospital, Rheumatology, Amiens, France*
[d]*Lille University Hospital, ULR 2694 - METRICS: Évaluation des technologies de santé et des pratiques médicales, Lille, France*
[e]*CHU de Rennes- Univ Rennes- INSERM- INRA- Institut NUMECAN Nutrition Metabolisms and Cancer- UMR 1241, Rheumatology, Rennes, France*
[f]*Regional Hospital of Orleans- EA 4708 – I3MTO- University of Orleans, Rheumatology, Orleans, France*
[g]*INSERM U1153 APHP Centre- Université de Paris, Rheumatology, Paris, France*

Background/Introduction: Reports of Elevated bone mass (EBM) on routine Dual energy X-Ray Absorptiometry (DXA) scanning are not infrequent. However, epidemiological studies of EBM are few and definition thresholds variable.

Purpose: The purpose of this French multicentric study was to evaluate the prevalence and causes of EBM in adult patients who underwent DXA scanning over a 10-year period.

Methods: This multicentric, retrospective study was conducted in six French regional bone centres. DXA databases were initially searched for individuals with a bone mineral density (BMD) Z-score ≥+4 at any site in the lumbar spine or hip from April 1st, 2008 to April 30st, 2018.

Results: In all, 72,225 patients with at least one DXA scan were identified. Of these, 909 (322 men and 587 women) had a Z-score ≥+4, i.e. a prevalence of 1.26% [1.18%-1.34%]. The DXA scan reports and imagery and medical records of the 909 EBM patients were reviewed and 936 causes were found. In 42 patients (4%), no cause could be determined due to unavailability of data. Artefactual causes of EBM were found in 752 patients (80%), in whom the predominant cause was degenerative disease of the spine (613 patients, 65%). Acquired causes of focal EBM – including Paget's disease (n=7) – were found in 12 patients (1%), and acquired causes of generalized EBM – including renal osteodystrophy (n=32), haematological disorders (n=20) and hypoparathyroidism (n=15) – in 84 patients (9%). Other causes were rare hereditary diseases and unknown EBM in 19 (2%) and 27 (3%) cases respectively.

Conclusion(s): The prevalence of EBM was approximately 1 in 100. These findings suggest that degenerative disease of the spine is the main cause of EBM, but that acquired or hereditary diseases are also causal factors.

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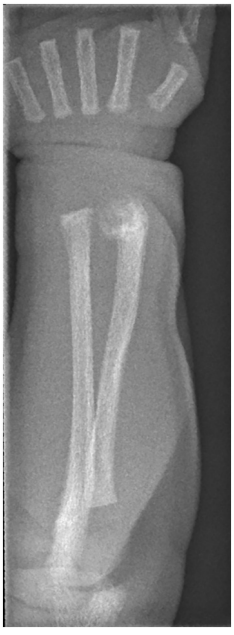
P176

Severe courses of neonatal FHH in paternally inherited CaSR mutations: Implications for perinatal monitoring and treatment

Jakob Höppner[a], Patricia Kutz[b], Claudia Roll[b], Wolfgang Högler[c], Corinna Grasemann[a]
[a]*St Josef-Hospital Bochum- Ruhr-University Bochum, Department of Pediatrics, Bochum, Germany*
[b]*Vestische Kinder- und Jugendklinik Datteln- Witten/Herdecke University, Department of Neonatology- Pediatric Intensive Care Medicine and Sleep Medicine, Datteln, Germany*
[c]*Johannes Kepler University Linz- Kepleruniversitätsklinikum, Univ.-Klinik für Kinder- und Jugendheilkunde, Linz, Austria*

Background/Introduction: Familial hypocalciuric hypercalcemia (FHH) is thought to be a benign endocrine condition with elevated PTH and resulting elevated calcium levels due to inactivating mutations in the calcium sensing receptor (CaSR). FHH can be distinguished from primary hyperparathyroidism by the low calcium excretion with the urine.

Methods: The neonatal courses of two siblings with FHH, one born after 25+6 gestational weeks and the other born full term are described.



Results: In the full term infant severe hyperparathyroidism, muscular hypotonia, thrombocytopenia, failure to thrive and multiple metaphyseal fractures (Figure 1) were observed and treatment with cinacalcet initiated. Both siblings inherited the paternally derived *CaSR* mutation [c.554G>A; p.(Arg185Gln)].

Conclusion(s): Few reports have described a severe clinical outcome in neonates with FHH due to paternally inherited mutations. Due to the differing calcium needs of the maternal and fetal organism severe hyperparathyroidism develops in the fetus and may result in severe perinatal complications as described in this report.

These cases indicate the necessity of a closer monitoring of calcium metabolism during pregnancy and counseling regarding the birth modus in cases of known paternal FHH. In addition, genetic counseling of affected male individuals should address potential perinatal complications.

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P013

Water bound to hydroxyproline is correlated with post-yield mechanical properties of human cortical bone

Mustafa Unal
Karamanoglu Mehmetbey University, Department of Mechanical Engineering, Karaman, Turkey

Background/Introduction: Water is the third major component of bone with forming ~ 10% of cortical bone's wet weight. Yet, its role in bone mechanical properties has not been fully understood. Though recent findings show significant associations between total water amount and the mechanical properties, such findings focused on total water amount without differentiating the possible role of different water fractions (i.e., free water, collagen-bound water, and mineral-bound water). We recently established a novel method to probe different water fractions in bone using Raman spectroscopy and identified three collagen-related water peaks and one mineral-related water peak in the Raman spectrum of bone at a high-wavelength region

Purpose: The aim of this study was to investigate a possible association between water bound to hydroxyproline (Hyp) amino acid of collagen and post-yield mechanical properties of human cortical bone.

Methods: 16 rectangular bone specimens were prepared and were broken under a three-point bending test to calculate post-yield mechanical properties of bone (i.e., post-yield toughness (PYT) and post-yield strain (PYS)). Raman spectra of bone were collected using a customized Raman spectroscopy. The intensity of the Raman peak located at ~ 3453 cm^{-1} normalized to organic matrix-related peak at ~ 2950 cm^{-1} was used to estimate the amount of water specifically bound to Hyp.

Results: The results showed that there were significant correlations between Hyp-bound water amount and PYT ($r = 0.62$, $p<0.05$) and PYS ($r = 0.59$, $p<0.05$), indicating that higher Hyp-bound water amount might be an indicator for better post-yield mechanical properties of bone.

Conclusion(s): Being a major component of bone's collagen, and playing an essential role in the stability of the collagen triple helix, the correlations between Hyp-bound water and post-yield mechanical properties might indicate a key role of water bound to Hyp in bone's mechanical competence. Yet, a causal link still needs to be established.

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P111

Skin autofluoresence, a non-invasive biomarker of advanced glycation end-products (AGEs), is associated with frailty: The Rotterdam study

Komal Waqas[a], Jinluan Chen[b], Katerina Trajanoska[b], Arfam Ikram[c], Fernando Rivadeneira[d], Trudy Voortman[c], M. Carola Zillikens[a]
[a]*Erasmus University Medical Center, Bone Centre- Department of Endocrinology and Internal Medicine, Rotterdam, Netherlands*
[b]*Erasmus University Medical Center, Department of Internal Medicine and Epidemiology, Rotterdam, Netherlands*
[c]*Erasmus University Medical Center, Department of Epidemiology, Rotterdam, Netherlands*
[d]*Erasmus University Medical Center, Department of Internal Medicine, Rotterdam, Netherlands*

Background/Introduction: We recently found that higher skin AGEs are associated cross-sectionally with sarcopenia, which precedes physical frailty. However, 82% of the subjects in our cohort with sarcopenia were not physically frail. A gold standard biomarker to evaluate frailty is lacking in clinical practice.

Purpose: To investigate the association between skin AGEs and frailty, a hallmark of ageing, measured both as Physical Frailty and through its multidimensional variant including cognitive and psychosocial aspects, the Frailty Index.

Methods: Cross-sectional analysis in 2521 Rotterdam study participants aged 45 years and older with assessment of skin AGEs as skin autofluorescence (SAF) using the AGE reader since 2012. Fried's frailty criteria was used to define Physical Frailty (presence of ≥3 components) and pre-frailty (1 or 2 components) including weight loss, muscle weakness, gait speed, exhaustion and decreased physical activity,. Rockwood's concept of Frailty Index was used including 38 deficits originating from categories such as functional status, cognition, diseases, health conditions, nutritional status and mood. Multinomial logistic and linear regressions were used with SAF as exposure and frailty as outcome adjusting for age, sex, Rotterdam study cohorts, renal function, diabetes, socioeconomic and smoking status.

Results: Mean SAF was 2.39 ± 0.49 arbitrary unit and median age was 74.2 years. Regarding Physical Frailty, 96 persons (4%) were frail and 1221 (48%) pre-frail; SAF was associated with both being pre-frail [odds ratio (95% confidence interval) = 1.29 (1.07 – 1.56)] and frail [1.87 (1.20 – 2.90)] after adjustments with non-frail subjects as reference. Regarding Frailty Index, the mean value was 0.14 (range 0-1) and higher SAF was associated with a higher frailty index [$\beta = 0.116$, $p = 1.3 \times 10^{-8}$].

Conclusion(s): Higher skin AGEs are associated with both Physical Frailty and Frailty Index in our cohort. Replication of our findings and longitudinal studies are needed to evaluate potential use of SAF as a biomarker of frailty.

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P107

The impact of Nordic walking on bone properties in postmenopausal women with pre-diabetes and non-alcohol fatty liver disease

Shenglong Le[a], Xiaming Du[b], Chao Zhang[c], Xiangqi Zhang[d], Zhen Qi[e], Shulin Cheng[a]
[a]*Shanghai Jiao Tong University, School of Life Sciences and Biotechnology- Exercise Translational Medicine Center- Shanghai Center for Systems Biomedicine, Shanghai, China*
[b]*Shidong Hospital, Department of Orthopedic, Shanghai, China*
[c]*Xidu Community Health Service Center of Fengxian District, Xidu Community Health Service Center of Fengxian District, Shanghai, China*

[d]Shanghai Jiao Tong University, School of Life Sciences and Biotechnology, Shanghai, China
[e]Shanghai Jiao Tong University, Exercise Translational Medicine Center, Shanghai, China

Background/Introduction: Regular physical exercise has been recommended as an effective and safe non-pharmacological strategy to maintain bone health.

Purpose: To investigate the impact of Nordic walking on bone properties in postmenopausal women with pre-diabetes and non-alcohol fatty liver disease (NAFLD).

Methods: The study is a part of a large randomized control trial. Of the eligible participants, 63 postmenopausal women (aged 50 - 65 years) with prediabetes and NAFLD participated in either a progressive supervised Nordic walking training (60 - 75% VO2max intensity) was given 2 - 3 times/week in 30 - 60 min/sessions for 8.6-month (the exercise group: AEx, n = 33), or maintained their daily lifestyle during the intervention (the control group: Con, n = 30). Pre- and post-intervention BMC and BMD of the whole body (WB), total femur (TF), femoral neck (FN) and lumbar spine (L2-4) were assessed by a dual-energy X-ray absorptiometry. Venous blood samples were analyzed for serum osteocalcin, pentosidine and receptor activator of nuclear factor kappa-B ligand (RANKL) levels.

Results: After 8.6-month intervention, the AEx group maintained their BMC_{TF}, BMD_{TF}, $BMC_{L2\text{-}4}$ and $BMD_{L2\text{-}4}$, and increased their BMC_{FN} ($p < 0.016$), while the Con group decreased their BMC_{TF} ($p < 0.008$), BMD_{TF} ($p < 0.001$)) and $BMD_{L2\text{-}4}$ ($p < 0.002$). However, no significant group × time interaction was observed in BMC and BMD, except for $BMD_{L2\text{-}4}$ ($p = 0.013$, partial $\eta^2 = 0.106$). Decreased pentosidine was correlated with increased BMC_{WB} ($r = -0.352$, $p = 0.019$). The intervention has no significant effect on the bone biomarkers of osteocalcin, and RANKL.

Conclusion(s): Our results suggest that Nordic walking is effective in preventing bone loss among postmenopausal women with pre-diabetes and NAFLD. Changing of bone mass is associated with changing of pentosidine. However, this effect is not associated with osteocalcin and RANKL.

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Concurrent Oral Poster Presentations 2: Basic / Translational: Bone Biology, Growth and Regeneration

P050

Pharmacological manipulation of early zebrafish skeletal development shows an important role for Smad9 in control of skeletal progenitor populations

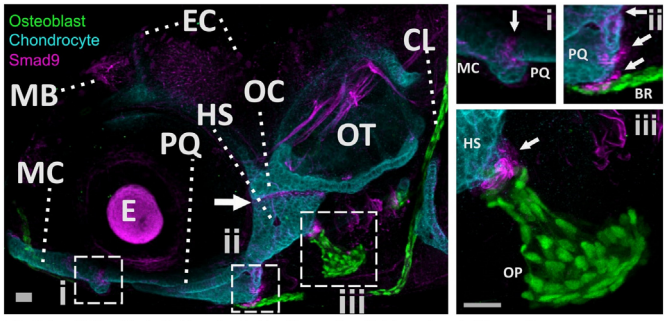
Georgina McDonald, Mengdi Wang, Chrissy Hammond, Dylan Bergen
University of Bristol, School of Physiology- Pharmacology- and Neuroscience, Bristol, United Kingdom

Background/Introduction: Genes associated with high bone mass (HBM) represent interesting targets for manipulation, as they could offer ways to increase bone density. A damaging p.L22P mutation in *SMAD9* has recently been associated with HBM. SMAD9 is a transcriptional repressor in the BMP signalling pathway and could function as a potential novel osteoanabolic osteoporosis drug target.

Purpose: We provide functional data of Smad9 expression focussing on the developing zebrafish skeleton and how Smad9 responds to drug treatments known to affect skeletal progenitor cell behaviour (prednisolone and retinoic acid (RA)) or inhibit BMP-signalling (dorsomorphin).

Methods: Protein expression was visualised with α-Smad9, α-collagen2a1 and α-GFP. Zebrafish transgenic reporters visualised osteoblasts (*sp7:GFP*), BMP-pathway (*BMPre:GFP*), and neural crest (*sox10:GFP*). Three days post-fertilisation (dpf) larvae were treated with 0.01% DMSO, 1μM RA, 4μM dorsomorphin or 25μM prednisolone until 5dpf. 6-hour treatment with 5μM nifurpirinol was used to ablate osteoblasts in *sp7:mCherry-NTR* transgenics. Experiments were ethically reviewed under UK Home Office licence (30/3801).

Results: Smad9 labels groups of osteochondral precursor cells at joint symphyses and at bone-cartilage interfaces such as the operculum (see image). This $Smad9^+$ pocket at the operculum expands following osteoblast ablation (1.74-fold, $p<0.01$) followed by proliferation osteoblasts. Treatment with RA and prednisolone altered Smad9 expression in craniofacial elements but dorsomorphin did not. RA treatment completely prevented osteoblast repopulation after ablation ($p<0.0001$).



Lateral view of 4 day old zebrafish larva showing Smad9 expression at joint symphyses (white arrow).
BR, branchiostegal ray; CH, ceratohyal; CL, cleithrum; E, eye; EC, epidermal cell; EP, ethmoid plate ; HS, hyosymplectic cartilage; MB, mid brain; MC, Meckel's cartilage; OC, otic cartilage; OP operculum; OT, otolith; PQ, palatoquadrate . Scale bar is 50 μm.

Conclusion(s): These results demonstrate that Smad9+ cells represent an undifferentiated osteochondral precursor population, which can be manipulated by commonly used skeletal drugs. We conclude that HBM associated Smad9 represents a target for future therapeutic targeting.

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P045

Inhibition of hypoxia-induced Mucin 1 alters proteomic composition of human osteoblast-produced extracellular matrix, leading to reduced osteogenic and angiogenic potential

Pavitra Kumar[a,b], Shuang Zhang[b], Marijke Koedam[b], Jeroen Demmers[c], Suvro Chatterjee[d], Johannes van Leeuwen[b], Bram van der Eerden[b]
[a]*University of Berne, Clinical Research, Berne, Switzerland*
[b]*Erasmus MC, Internal Medicine, Rotterdam, Netherlands*
[c]*Erasmus MC, Proteomics, Rotterdam, Netherlands*
[d]*Anna University, Biotechnology, Chennai, India*

Background/Introduction: The extracellular matrix (ECM) creates a physical framework for cells in bone that facilitates cellular events such as adhesion, differentiation, migration, proliferation and survival. We previously found indications that Mucin 1 (MUC1) was able to modify hypoxia-induced upregulation of collagens and disrupted mineralization by human osteoblasts.

Purpose: We assessed the MUC1-mediated effect of hypoxia on proteomic composition of human osteoblast-derived ECMs and characterize their osteogenic and angiogenic potentials.

Methods: Human bone marrow-derived mesenchymal stromal cells (MSCs) were osteogenically differentiated into osteoblasts under normoxia (20% O_2) or hypoxia (2% O_2) with or without 5 μM GO-201 (MUC1 inhibitor) and osteogenic markers were measured by biochemical assays, gene expression and immunoblotting. On day 11, osteoblasts were devitalized using freeze-thawing cycles and DNase treatment. Proteomic composition of ECMs was analyzed using LCMS with a label-free quantification (LFQ) method. ECMs were used for subsequent MSCs or Human umbilical vein endothelial cell (HUVECs) cultures.

Results: HIF1α levels were increased on day 1 and day 6 by 3-fold ($p<0.05$) and 2.5-fold ($p<0.05$) respectively, in hypoxic cells compared to cells under normoxic conditions. MUC1-CT inhibition under 2% oxygen reduced HIF1α levels on day 1 by 3-fold ($p<0.05$) compared to untreated cells. Hypoxia and/or MUC1-CT inhibition reduced matrix mineralization by human osteoblasts through the AMPK/mTORC1/phospho-S6 pathway. Proteomic analysis revealed 3,182 proteins shared by all four ECMs (≥ 2 detected peptides), with specific proteins up- and downregulated by hypoxia, GO-201 or the combination compared to normoxic ECMs. Hypoxia modulated ECMs by TGFβ/Smad and phosphorylation of NFκB (1.8-fold, $p<0.05$). ECMs of hypoxic osteoblasts were pro-osteogenic, whereas ECMs of MUC1-CT inhibited-osteoblasts showed reduced osteogenic and angiogenic properties.

Conclusion(s): We claim here that MUC1 is critical for hypoxia-mediated changes during osteoblastogenesis, which not only alters the proteomic landscape of the ECM but thereby also modulates its osteogenic and angiogenic potentials.

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P052

Reciprocal protein stabilization of ABL and TAZ regulates osteoblastogenesis and embryonic bone development through transcription factor RUNX2

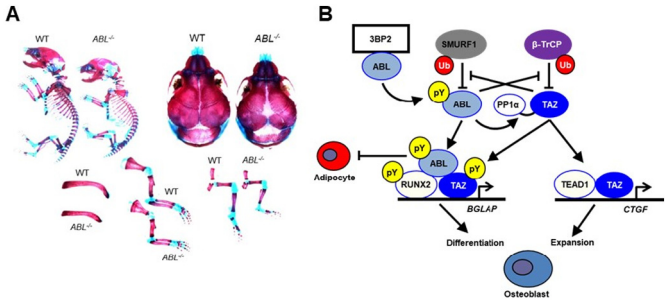
Yoshinori Matsumoto[a], Robert Rottapel[b]
[a]*Okayama University Graduate School of Medicine- Dentistry and Pharmaceutical Sciences, Department of Nephrology- Rheumatology-Endocrinology and Metabolism, Okayama, Japan*
[b]*Princess Margaret Cancer Center, Department of Immunology, Toronto, Canada*

Background/Introduction: Cellular identity in metazoan organisms is frequently established through lineage-specifying transcription factors, which control their own expression through transcriptional positive feedback while antagonizing developmental networks of competing lineages.

Purpose: In distinction to this model, we have uncovered a unique positive feedback loop, which arises from the reciprocal stabilization of ABL and TAZ protein required for osteoblast differentiation and embryonic skeletal formation.

Methods: We examined the calvarium and limb bones from newborn pups by micro-CT and histomorphometric analysis. To investigate the molecular mechanism, we isolated and cultured murine calvarial osteoblasts from *Sh3bp2*$^{-/-}$,*Smurf1*$^{-/-}$,*Abl*$^{fl/fl}$, *Abl*$^{-/-}$ and TAZ-deficient mice for *in vitro* study. Co-IP, ChIP, pulse chase, luciferase assay, BrdU assay, ubiquitin assay, cell growth assay and differentiation assay were performed in this study.

Results: We found a unique positive feedback loop between the tyrosine kinase ABL and the transcriptional co-activator TAZ. During osteoblastogenesis, ABL and TAZ reciprocally stabilized each other at the protein level through the exclusion of their respective E3-ubiquitin ligases, SMURF1 and β-TrCP. Stabilized and active ABL potentiated the assembly and activation of the RUNX2-TAZ master transcription factor complex that is required for osteoblastogenesis and embryonic bone formation while antagonizing PPARγ-mediated adipogenesis. ABL also enhanced TAZ nuclear localization and the formation of the TAZ-TEAD complex that is required for osteoblast expansion. Lastly, we have provided genetic data showing that the regulation of the ABL-TAZ amplification loop lies downstream of the adaptor protein 3BP2, which is mutated in the craniofacial dysmorphia syndrome cherubism.



Conclusion(s): Our study demonstrates an interplay between ABL and TAZ that controls the mesenchymal maturation program towards the osteoblast lineage and is mechanistically distinct from the established model of lineage-specific maturation.

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P084

New insights into cells of human bone marrow adipose tissue

Drenka Trivanovic[a,b], Bianca Schlierf[a,b], Konstantin Horas[b,c], Janek Hader[b], Theresa Kreuzahler[a,b], Maximilian Rudert[b,c], Marietta Herrmann[a,b]
[a]*University Hospital Wuerzburg, IZKF Group Tissue Regeneration in Musculoskeletal Diseases, Wuerzburg, Germany*
[b]*University of Wuerzburg, Bernhard-Heine-Center for Locomotion Research, Wuerzburg, Germany*
[c]*University of Wuerzburg, Department of Orthopaedic Surgery- König-Ludwig-Haus, Wuerzburg, Germany*

Background/Introduction: Physiological aging and stress conditions may cause expansion of bone marrow (BM) adipose tissue (BMAT). Due to mechanical and paracrine activity, BMAT regulates skeletogenesis and bone health.

Purpose: Recent findings suggested complex composition of BMAT, while BMAT-residing cell phenotypes are not fully elucidated. Here, we bring novel evidences on BMAT niche cell peculiarities.

Methods: Acetabular BM reaming samples (n=19; female=11, male=8) were obtained from patients undergoing total hip arthroplasty. The research was approved by the local ethical committee (No. 187/18). BMAT and associated MNC were collected from the floating layer of BM samples, while BM mononuclear cells (BM-MNC) were isolated from the layer below. *Ex vivo* and *in vitro* assays were performed to analyze cells using flow cytometry, clonogenic assays and histochemical staining. Statistical significance was estimated by ANOVA. Non-parametric Spearman's rank correlation (r) was calculated.

Results: BMAT number of MNC/mL positively correlated with the age of patients (r=0,387; p=0,041, n=12). Frequency of colony-forming unit–fibroblastic was higher in BMAT-MNC (0,018±0,0098%)

than in BM-MNC (0,008±0,0023%, p=0,023). This was associated with higher incidence of $CD45^{-}CD31^{-}$ (non-hematopoietic and non-endothelial) cells within BMAT-MNC (85,5±22,21%) than in BM-MNC (45,24±19,23%). Accordingly, frequency of clonogenic hematopoietic cells was higher in BM-MNC (0,103±0,088%) in BMAT-MNC (0,045± 0,011%, p=0,042). Analyses of alive cells using neutral lipid and mitochondrial membrane potential probes, revealed reduced lipid content and mitigated metabolic activity of BMAT-MNC. We found lower percentage of $Bodipy^{high}$ cells within BMAT-MNC (7,65±4,53%) than BM-MNC (48,76±13,55%) and lower frequency of $Mito^{high}$ in BMAT-MNC (35,43±15,1%) than in BM-MNC (70,01±21,31%). Expanded BMAT stromal cells displayed similar osteogenic but stronger adipogenic potential than their BM counterparts.

Conclusion(s): Results indicate that BMAT might provide microenvironmental signals distinct to those in other BM niches, containing cell populations with specific metabolic profile and functional properties. Their understanding can improve strategies to target cells which contribute to BMAT expansion and skeletal diseases.

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P172

Depletion of odontoblasts induces dental tissue regeneration

Toshihide Mizoguchi
Tokyo Dental College, Oral Health Science Center, Tokyo, Japan

Background/Introduction: Reparative dentin formation is induced in response to severe dental damage. This is a biological defense mechanism to regenerate the damaged hard tissue. In this biological process, dental damage induces odontoblast death, after which dental pulp stem cells differentiate into odontoblast-like cells, contributing to reparative dentin. However, how damage triggers this regenerative process remain unclear.

Purpose: We hypothesized that odontoblast death triggers regeneration of damaged dental tissue. In this study, we examined the effects of odontoblastic depletion on dentinogenesis activation using a Cre/LoxP-based strategy.

Methods: (1) To induce cell death specifically in the odontoblasts using a Cre/LoxP-based strategy, we confirmed the odontoblast-specific expression pattern of green fluorescent protein (GFP) in type I collagen α *[(Col1 (2.3)]-GFP* mice, in which GFP is expressed under the control of a 2.3-kb fragment of *Col1* promoter. (2) *Col1 (2.3)-Cre; ROSA26-loxP-stop-loxP-diphteria toxin (DT) receptor (DTR); Col1(2.3)-GFP* mice were generated and administered DT for 1 week to deplete odontoblasts and analyzed the regeneration of odontoblasts and reparative dentin formation.

Results: (1) The expression of *Col1 (2.3)* promoter-inducible GFP was only detected in odontoblasts in the maxillary first molar, confirming that Col1 (2.3)-Cre was specifically expressed in odontoblasts. (2) Odontoblasts were dramatically depleted in maxillary first molars after DT treatment. (3) Depleted odontoblasts were significantly recovered in a time-dependent manner. (4) Regenerative odontoblast-like cells generated reparative dentin.

Conclusion(s): Dentin formation increased in response to odontoblastic cell death in a genetically modified mouse model. This suggests that there is a dental pulp niche environment regulated by odontoblastic cell death and that this regulatory network is essential for the activation of reparative dentin formation.

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P201

Studies of OI patient and murine osteoblasts to investigate phenotypic variability of dominant Osteogenesis Imperfecta

Milena Jovanovic[a], Apratim Mitra[b], Chris Stephan[c], Kenneth Kozloff[c], Ryan Dale[b], Joan C. Marini[a]
[a]*NICHD/NIH, Heritable Disorders of Bone and Extracellular Matrix, Bethesda, United States*
[b]*NICHD/NIH, Bioinformatics and Scientific Programming Core, Bethesda, United States*
[c]*University of Michigan, Department of Orthopaedic Surgery, Ann Arbor, United States*

Background/Introduction: 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.

Purpose: We present the first comparative study of osteoblast differentiation from normal pediatric controls vs OI patients with phenotypic variability. Additionally, we research phenotypic variability in OI mouse models using Brtl (*COL1A1* Gly349Cys) and a new Gly349Ser mice.

Methods: We focused on *COL1A1* mutations Gly352Ser and Gly589Ser, each in two unrelated patients differing in phenotypic severity.

Results: Patient OB cell layer and secreted collagen were overmodified shown by ^{3}H steady-state assay, indicating delayed folding; 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 severe patient OB depositing significantly less mineral than 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. Also, we study the effect of the collagen glycine substituting residue on murine skeletal phenotype, comparing a new Brtl(Ser) and our Brtl(Cys) mouse. Both mice are small but Brtl(Ser) has a more severe phenotype with rib fractures, limb bowing, flared ribcage, kyphosis by pups skeletal staining and undermineralization by BMD. Preliminary uCT data indicated severe decrease of Tb BV/TV in Brtl(Ser) 7-wk-femora. Brtl(Ser) calvarial osteoblasts have lower collagen secretion and lower mineral deposition than wt. Electron microscopy indicated Brtl(Ser) dermal collagen fibrils have altered packing and cross-section.

Conclusion(s): This combined human/mouse approach will lead to novel insights into OI osteoblast differentiation, roles of OB and matrix in phenotypic variability and the effect of substituting residue and position along the collagen helix.

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P082

Osteocalcin deficiency in a mouse model of severe dominant osteogenesis imperfecta rescues metabolic but not bone phenotype

Josephine Tauer[a,b], Mathieu Ferron[c,d], Svetlana Komarova[a,b]
[a]*McGill University, Dentistry, Montreal, Canada*
[b]*Shriners Hospital for Children, Shriners Hospital for Children, Montreal, Canada*
[c]*Institut de Recherches Cliniques de Montréal, Unité de Recherche en Physiologie Moléculaire, Montreal, Canada*
[d]*Université de Montréal, Départements de Médecine et de Biochimie et Médecine Moléculaire, Montreal, Canada*

Background/Introduction: The osteoblast-derived hormone osteocalcin (OCN) plays a significant role in regulating whole-body metabolism, muscle adaptation, and reproduction. Osteogenesis imperfecta, mainly caused by collagen-I gene mutations, is characterized by high bone turnover and low bone mass. Previously, we demonstrated that Col1a1Jrt/+ mice with a severe dominant form of OI displayed elevated OCN serum levels along with an altered glucose/insulin metabolism and energy expenditure, especially at a young age.

Purpose: To evaluate OCN's role in OI, we assessed bone and metabolic phenotype in Col1a1Jrt/+ mice (OI) with deficient (OI/OCN-/-) or reduced OCN levels (OI/OCN+/-).

Methods: OI/OCN mice were characterized for their growth phenotype, glucose metabolism, and bone properties at 4-, 8- and 12-weeks of age.

Results: For the first-generation, OI mice were crossed with fertile male OCN+/- and female OCN-/-. About 39% of generated mice were WT/OCN+/-, 29% OI/OCN+/-, 16% OI/WT or WT/WT, and 0.4% were found dead. For second-generation, OI/OCN+/- mice were intercrossed and gave birth to about 30% of pups with WT/OCN+/-, 20% OI/OCN+/-, 16% OI/OCN-/-, 12% WT/OCN-/-, 11% OI/WT, 4% WT/WT, and 7% were found dead. Compared to WT/WT, mice with OI genotype were smaller in size and up to 20% lower in body mass at all ages investigated. At 4-weeks of age, OI/WT, OI/OCN+/- and OI/OCN-/- mice exhibited significantly lower fasting glucose levels than WT/WT littermates. OI/OCN+/- mice, similar to OI/WT, demonstrated significantly improved glucose tolerance, while OI/OCN-/- mice did not differ from WT/WT. At older ages, no significant differences in fasting glucose or glucose tolerance were found in OI/WT, OI/OCN+/- or OI/OCN-/- mice compared to WT/WT. Assessment of femoral structural and mechanical properties revealed persistent bone fragility in OI/OCN+/- or OI/OCN-/- similar to OI/WT mice at all ages investigated.

Conclusion(s): These results strongly support the causative role of osteocalcin driving alteration in glucose/insulin metabolism in OI mice.

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P054

Using machine learning to automate image segmentation for analysis of in vitro osteoclast formation and bone resorption

B.K. Davies[a], Andrew Hibbert[a], Mark Hopkinson[a], Gill Holdsworth[b], Isabel Orriss[a]
[a]*Royal Veterinary College, Comparative Biological Sciences, London, United Kingdom*
[b]*UCB Pharma Ltd., New Medicines, Slough, United Kingdom*

Background/Introduction: Quantification of osteoclast culture endpoints (e.g., osteoclast numbers, bone area) often relies on manual analysis methods. Whilst this approach enables user confirmation of osteoclasts and associated pits, it is labour-intensive, extremely time-consuming and results in substantial user variability.

Purpose: This study aimed to develop and validate an automated, machine learning (ML)-based workflow to simultaneously, reliably and robustly quantify osteoclast culture endpoints.

Methods: Historic images of TRAP-stained mouse bone marrow-derived osteoclasts cultured on dentine discs were used to train the ilastik-based ML algorithm.

Results: Assessment of algorithmic training revealed that osteoclast numbers and the total area resorbed strongly correlated between manual- and automatically-quantified values ($r = 0.75$ and 0.83, respectively). Osteoclasts were faithfully segmented when visually compared to the original images. The accuracy of automated osteoclast number quantification was validated using Zoledronate, a bisphosphonate with well-characterised inhibitory effects on osteoclasts, and Ticagrelor, a $P2Y_{12}$ receptor antagonist with less well-known effects on osteoclast biology. A 70% reduction ($p<0.01$) in osteoclast number was detected, irrespective of quantification method, when cultured with 10nM Zoledronate. Both methods also detected a dose-dependent decrease in osteoclast number when treated with 1-10μM Ticagrelor ($p<0.05$). Development of the ilastik algorithm reduces user variability by ≤100% ($p<0.001$).

Resorption pits were occasionally inaccurately identified using the ilastik algorithm; therefore, microCT-based protocols quantifying bone resorption were also developed. Dentine discs with adherent osteoclasts were scanned at 2μm; resorption area was visualised and quantified using CTvox and CtAn, respectively. Three-dimensional reconstructions reveal significant reductions in osteoclast resorptive activity following Zoledronate treatment; automated and manual quantification values were comparable.

Conclusion(s): Overall, an automated image segmentation and analysis workflow was developed and validated to consistently and sensitively identify osteoclasts, but not resorption pits. MicroCT analysis could provide an alternative solution to automate bone resorption analysis. This pipeline significantly reduces user variability of endpoint measurements and analysis time by 75%.

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P083

Distinct phenotypes of bone fragility in patients with type 2 diabetes mellitus depending on the presence of vascular complications: A study of three-dimensional trabecular microarchitecture

Aleksandar Cirovic[a], Ana Cirovic[a], Marko Vujacic[b], Zoran Bascarevic[b], Danijela Djonic[a], Marija Djuric[a], Petar Milovanovic[a]
[a]*Faculty of Medicine- University of Belgrade, Institute of Anatomy, Belgrade, Serbia*
[b]*Faculty of Medicine- University of Belgrade, Institute for Orthopaedic Surgery "Banjica", Belgrade, Serbia*

Background/Introduction: Presence of micro- and macrovascular complications in individuals with diabetes mellitus type 2 (T2DM) increases hip fracture risk additionally.

Purpose: Although the majority of individuals with T2DM develop vascular complications during the lifespan, there are insufficient data about trabecular microarchitectural variations in the femoral neck depending on the presence of diabetic vascular complications.

Methods: We analyzed femoral neck trabecular microarchitecture via microcomputed tomography in 16 individuals with T2DM who underwent total hip arthroplasty due to hip fracture (13 women and 3 men, mean age: 78 ± 7 years). Seven individuals had vascular complications including coronary artery disease, stroke, or carotid disease (DMFx_VD group) and nine individuals did not present vascular complications (DMFx_NVD group). The following microarchitectural parameters were determined: bone volume fraction (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), structure model index (SMI), and trabecular pattern factor (Tb.Pf). We calculated FRAX score and conducted DXA measurement of the contralateral hip. Hemoglobin A1c (HbA1c) level was also determined. Institutional review board approved the study.

Results: Patient age, duration of disease, and value of HbA1c did not vary between the groups ($p=0.06$, $p=0.71$, $p=0.99$, respectively). T-score was significantly reduced ($p=0.02$), while FRAX value for hip fracture ($p=0.03$) was increased in the DMFx_VD group. Compared with the DMFx_NVD group, DMFx_VD group had a lower BV/TV ($p=0.047$), decreased Tb.N ($p=0.02$) and increased Tb.

Sp (p=0.004), while SMI, Tb.Th, and Tb.Pf did not vary between the groups (p=0.16, p=0.82, p=0.052, respectively).

Conclusion(s): Two distinct phenotypes of bone fragility were identified in T2DM patients. Namely, patients with vascular complications showed impaired trabecular microarchitecture compared with individuals without vascular complications. Such heterogeneity may set a basis for further basic and clinical studies related to T2DM fracture risk.

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P148

MicroRNAs in exosomes as circulating biomarkers for postmenopausal osteoporosis with fragility fractures

Qun Cheng
Huadong Hospital affiliated to Fudan University, Department of Osteoporosis and Bone Disease, Shanghai, China

Background/Introduction: Postmenopausal osteoporosis (PMOP) brings a heavy burden to society and seriously affects the quality of life of elderly people. Circulating biomarkers based on microRNAs (miRNAs), especially exosomal miRNAs, are widely studied.

Purpose: The purpose of this study was to identify specific miRNAs in exosomes from the serum of patients suffering from PMOP with fragility fractures and to validate these molecules as novel biomarkers.

Methods: Postmenopausal women with osteoporotic fracture and normal bone mass were selected from the community. Based on microarray analysis, we compared the alterations in circulating exosomal miRNAs between the 2 groups. Target gene pathway enrichment of diverse miRNAs was analyzed by the online bioinformatics tools TargetScan and DAVID. Furthermore, the ALP activities of BMSCs regulated by miRNAs associated with bone mineral density (BMD) were studied by transfecting these miRNAs into human bone marrow mesenchymal stem cells (hBMSCs).

Results: A total of 239 miRNAs were found to be significantly expressed (P<0.05). Three miRNAs (miR-324-3p, miR-766-3p and miR-1247-5p) were found to be associated with both BMD parameters, while miR-330-5p and miR-3124-5p were found to be associated with BMD of the hip. Potential roles of 5 miRNA in Wnt signaling pathway were analysed. Remarkably, miR-330-5p was found to promote the ALP activity of hBMSCs, while mir-3124-5p showed the opposite result.

Conclusion(s): This study identified differentially expressed miRNAs in PMOP with fragility fractures. MiR-324-3p, miR-766-3p, miR-1247-5p, miR-330-5p, and miR-3124-5p, which were associated with BMD, could be biomarkers for the diagnosis of PMOP with fragility fractures and provide future research directions.

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P161

Sequential dosing with zoledronate following OPG:Fc treatment protects against rebound bone loss

Jad Zamerli[a], Sindhu Mohanty[a], Ya Xiao[a], Albert Kim[b], Jacqueline Center[c], Christian Girgis[b], Peter Croucher[a], Michelle Mcdonald[a]
[a]*Garvan Institute, Bone Biology, Darlinghurst, Australia*
[b]*Royal Northshore Hospital, Endocrinology, St Leonards, Australia*
[c]*Garvan Institute, Bone, Darlinghurst, Australia*

Background/Introduction: Denosumab (Dmab) cessation results in rebound bone loss and increased fracture risk. Elevated osteoclast activity underlies this phenomenon, providing the potential to mitigate bone-loss through inhibiting osteoclasts.

Purpose: We hypothesized that sequential bisphosphonate therapy would prevent Dmab induced rebound bone loss, and that a multi-dose strategy would be superior.

Methods: We compared single-dose zoledronate (OPG-SDZA) and double-dose (OPG-DDZA) after 2 weeks of OPG:Fc (10mg/kg) treatment and compared this to OPG:Fc alone (OPG-A). OPG-SDZA (0.01mg/kg) was administered at week 5 only, and OPG-DDZA at weeks 5 and 12.

Results: Despite OPG:Fc treatment ceasing at week 2, bone mineral density (BMD) peaked at week 10, with all OPG treatment groups increased 21-22% compared to vehicle (p<0.0001). By week 14 a 15% reduction in BMD normalised OPG-A treated mice to vehicle levels, whereas only a 6% reduction occurred in OPG-SDZA mice, maintaining higher BMD than OPG-A (p<0.001). Importantly, BMD was not altered form 10-17 weeks in OPG-DDZA treated mice, maintaining 7-10% and 18-24% higher BMD than OPG-SDZA and OPG-A respectively (p<0.01).

Serum TRAP remained reduced versus vehicle at week 10 in all OPG:Fc treated groups (p<0.001). 2 weeks later, and prior to BMD reductions, serum TRAP rose rapidly in OPG-A mice, reaching 68% higher than vehicle (p<0.0001). Conversely, serum TRAP levels in mice treated with ZA (single and double dose) rose to reach vehicle levels at week 10 and remained equivalent to vehicle.

Micro-CT data of trabecular bone 15-weeks post OPG:Fc cessation revealed 3 and 4.5 fold increases in BV/TV with OPG-SDZA and OPG-DDZA compared to OPG-A respectively (p<0.0001). Cortical bone volume was increased in OPG-DDZA group 9% and 8% compared to both OPG-A and OPG-SDZA respectively (p<0.05).

Conclusion(s): Our data demonstrates that sequential ZA treatment mitigates OPG:Fc cessation induced rebound bone-loss through supressing rebound bone resorption, with multiple ZA doses more effective than a single dose.

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NI Seminar

P168

The effect of three different vitamin D3 supplementation regimens in deficient subjects – a randomized open-label parallel group study

Angelo Fassio, Giovanni Adami, Ombretta Viapiana,
Alessandro Giollo, Rossini Maurizio, Gatti Matteo, Davide Gatti
Rheumatology Unit, University of Verona, Italy

Background/Introduction: Currently, recommendations for vitamin D supplementation vary between scientific societies, and the best regimen to treat deficient patients is still not clear [1].

Purpose: The aim of our study was to compare the pharmacokinetic profile of three different regimes of cholecalciferol supplementation in terms of 25OHD exposure and safety profiles.

Methods: Healthy subjects with vitamin D deficiency (defined as 25(OHD)<20 ng/mL), 18 to 60 years of age were enrolled and randomized into three different arms: daily 10,000Iu for 8 weeks, weekly 50,000Iu for 12 weeks and biweekly 100,000Iu for 12 weeks. Serum 25OHD was dosed weekly in all groups. 25OHD was measured by the IDS-ISYSMulti-Discipline automated analyser (Immunodiagnostic System, Boldon, UK) based on chemiluminescence technology. The CV intra-assay measured in our laboratory was 6% (inter-assay CV 9%). The study was authorized by the local ethical committee (protocol DIBA/11. Supported by Abiogen Pharma, Italy).

Results: A total of 75 subjects were enrolled. The descriptive of the sample at baseline and relative 25(OH)D levels at the various observation points are reported in table 1. Mean 25(OH)D plasma levels significantly increased to peak values of 81.0±15.0 ng/mL in Group A, 63.6±7.9 ng/mL in Group B and 59.4±12 ng/mL in Group C. The percentage of patients achieving serum 25(OH)D levels ≥ 20 ng/mL and ≥ 30 ng/mL is shown in figure 1. The comparison of the 25(OH)D areas under the curve (AUCs) expressing the systemic exposure to 25(OH)D levels in the three different groups is shown in figure 2. No serious adverse event occurred.

Table 1: mean values ± SD at the different observation points.

Parameter	Daily 10.000 Ui (N = 25)	Weekly 50.000 Ui (n = 25)	Biweekly 100.000 Ui (N = 25)	p-value (ANOVA)
M:F	12:13	7:18	12:13	
Age (years)	30.2 ± 9.9	36.7 ± 8.7	35.4 ± 11.0	*NS
Body Weight (kg)	65.8 ± 13.2	67.8 ± 10.8	66.6 ± 13.7	*NS
Height (m)	1.7 ± 0.1	1.68 ± 0.1	1.7 ± 0.1	*NS
BMI	22.55 ± 2.7	23.8 ± 2.2	22.8 ± 2.7	*NS
Baseline 25OHD (ng/mL)	14.6 ± 3.9	12.8 ± 3	13.5 ± 4.1	*NS

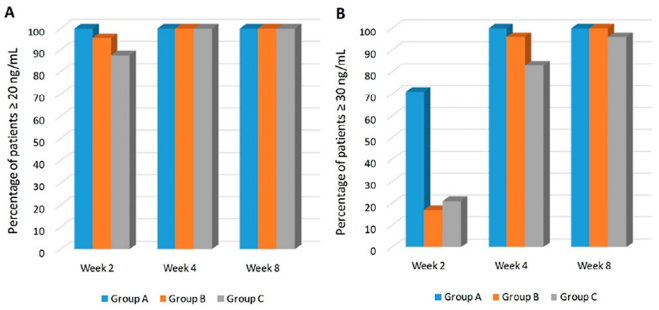

Figure 1: Percentage of patients achieving the 20 ng/mL (A) and 30 ng/mL threshold (B) at weeks 2, 4,and 8. Group A; 10,000 IU daily, Group B; 50,000 IU weekly and Group C; 100,000 IU bi-weekly doses of cholecalciferol

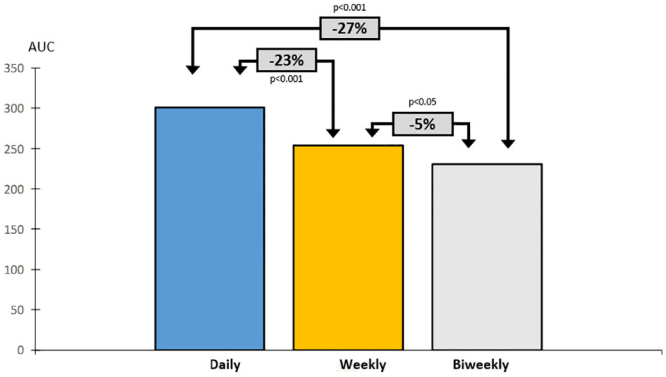

Figure 2: AUC of 25(OH)D serum levels at 12th weeks of treatment

Conclusion(s): All the three different regimens proved to be effective in correcting vitamin D deficiency already after 1 months (2 weeks for the daily regimen). However, the daily regimen showed a higher efficacy than the bolus-based regimens. The safety profile was excellent in all groups.

Reference: [1] R. Bouillon, Comparative analysis of nutritional guidelines for vitamin D, Nat. Rev. Endocrinol. 13 (2017) 466–479. https://doi.org/10.1038/nrendo.2017.31.

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2352-1872/$ – see front matter

P042

A multi-omics approach to decipher adipocyte-induced transdifferentiation of osteoblast

Federica Quacquarelli, Ayyoub Salmi
ULCO, MAB lab, Boulogne-sur-Mer, France

Background/Introduction: Our preliminary findings lead us to propose bone marrow adipocyte secretions as new contributors to bone loss. Indeed using an *in vitro* coculture model based on human bone marrow stromal cells, we previously showed that soluble factors secreted by adipocytes induced the conversion of osteoblasts towards an adipocyte-like phenotype. This change in fate was confirmed by microarray gene expression profiling showing an enrichment in the adipocyte gene signature in cocultured osteoblasts. Furthermore double immunofluorescence staining demonstrated the co-expression of adipogenic and osteoblast specific markers in individual cells, providing evidence for a transdifferentiation event.

Purpose: The aim of this study was to identify adipocyte-secreted factor(s) implicated in the transdifferentiation of osteoblasts into adipocyte-like cells, and the molecular mechanisms underlying their actions.

Methods: One dimensional LC-MS/MS and label-free quantification were used to compare stimulatory and non-stimulatory adipocyte culture supernatants and identify a set of adipocyte-secreted proteins potentially regulators of osteoblast differentiation. In parallel, gene expression changes induced by adipocyte secretion products in the osteoblastic cells were identified by transcriptional analysis at two early stages of coculture. Physical interactions between adipocyte secreted proteins and osteoblast membrane protein coding genes were then investigated using data from StringDB.

Results: The network of interactions consisted of 92 adipocyte-secreted proteins interacting with 454 osteoblast membrane protein coding genes, for a total of 271 predicted physical interactions. By linking these results with genes whose expression was subsequently modified in cells, we identified potential pathways affected by the coculture. For the validation of our approach, we focused on some of them, including TGF-beta/Smad and PI3K/AKT signalling pathways, of particular interest in the balance between osteogenesis and adipogenesis.

Conclusion(s): Our findings provide new insights into the crosstalk between adipocytes and osteoblasts in the bone marrow and demonstrate the powerfulness of our integrative omics strategy to decipher cell-cell communication events.

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P002

Vitamin D levels in type 1 diabetes with and without neuropathy; A case-control study

Tatiane Vilaca[a], Fatma Gossiel[a], Dinesh Selvarajah[b], Richard Eastell[a]
[a]*University Of Sheffield, Academic Unit of Bone Metabolism, Sheffield, United Kingdom*
[b]*University Of Sheffield, Oncology and Metabolism, Sheffield, United Kingdom*

Background/Introduction: Type 1 diabetes is associated with increased risk of fractures, especially in patients with neuropathy. Diabetic neuropathy has been associated with low vitamin D levels, but the role of $1,25(OH)_2$ vitamin D has not been investigated.

Purpose: To investigate differences in $1,25(OH)_2$ vitamin D levels and its regulators in participants with type 1 diabetes with and without neuropathy and controls.

Methods: We compared patients with T1D with (T1DN+, n=20) and without (T1DN-, n=20) distal symmetric sensorimotor polyneuropathy, and controls (n=20). Distal symmetric sensorimotor polyneuropathy was defined by the Toronto Clinical Neuropathy Score and nerve conduction measured by DPN check. We measured 25(OH) vitamin D, $1,25(OH)_2$ vitamin D, PTH and albumin using Sandwich Chemiluminescence ImmunoAssay (CLIA), vitamin D binding protein and intact FGF-23 using ELISA.

Results: $1,25(OH)_2$ vitamin D was lower in T1DN+ and T1DN- compared to controls (44.7 ±9.4; 56.9 ±16.7; 81.6 ±25.6 pg/mL). 25 (OH) vitamin D was lower in T1DN+ compared to controls [20.0 ng/mL (10.9, 31.1) and 32.6 (22.5,41.5) p=0.010). There were no differences in albumin, vitamin D binding protein, PTH or intact FGF-23 between groups. $1,25(OH)_2$ vitamin D correlated negatively with Toronto Clinical Neuropathy Score (r=-0.458 p=0.003) and positively with sural nerve conduction velocity measured by DPN check (r=0.369 p=0.02).

Conclusion(s): T1D with neuropathy is associated with low 1,25D that may be due to low substrate (25(OH)D) but not to low binding proteins or PTH or high intact FGF-23. The low 1,25(OH)2 vitamin D is associated with measures of neuropathy.

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P055

Extracellular pH is a critical regulator of osteoclast fusion, size and activation

B.K. Davies[a], Mark Hopkinson[a], Gill Holdsworth[b], Tim Arnett[c], Isabel Orriss[a]
[a]*Royal Veterinary College, Comparative Biological Sciences, London, United Kingdom*
[b]*UCB Pharma Ltd., New Medicines, Slough, United Kingdom*
[c]*UCL, Division of Biosciences, London, United Kingdom*

Background/Introduction: Extracellular pH is a known modulator of osteoclast function. Acidosis directly stimulates bone resorption with near-maximal effects at pH7.0, whilst ≥pH7.4 osteoclast resorptive activity is very limited.

Purpose: This study investigated the mechanistic actions of low pH on osteoclast fusion and resorption.

Methods: Mouse bone marrow-derived osteoclasts were cultured on dentine discs at pH7.4 or pH6.9 for 5 days. Osteoclast formation and resorptive activity were measured by image analysis of TRAP-stained discs. The effect of pH on gene and protein expression and nuclei number was investigated using qPCR, western blotting and immunofluorescence, respectively.

Results: Osteoclast number was ≤1.9-fold ($p<0.001$) higher in cells cultured at pH6.9 compared to pH7.4 osteoclasts. Extensive resorptive activity was observed in pH6.9-cultured osteoclasts; the level of resorption in pH7.4 osteoclasts was 80% lower ($p<0.001$). Culture at pH7.4 was associated with the formation of larger, more nucleated osteoclasts (≤140μm with 23 ± 15 nuclei/cell), whereas cells at pH6.9 were ≤35μm in size with 8 ± 3 nuclei/cell. mRNA expression of osteoclast formation (e.g., RANK, c-FMS, TRAF6) and resorption genes (e.g., cathepsin K, carbonic anhydrase II) was decreased in pH7.4 osteoclasts. At the protein-level, pH7.4 osteoclasts unexpectedly expressed 1.5-fold more cathepsin K that was ~10% more active compared to resorbing pH6.9 osteoclasts ($p<0.01$). Protein expression of the fusion marker DC-STAMP was 1.5-fold greater in pH7.4-cultured osteoclasts compared to pH6.9; no differences were observed at the mRNA-level.

Short-term acid exposure of pH7.4 osteoclasts reduced osteoclast size by 40% 4-hours post-acidification, and increased osteoclast numbers by ≤90%. Resorptive activity is visible at 8-hours and is extensive 24-48-hours post-acidification. Acidification of osteoclasts originally cultured at pH7.4 results in a near similar resorptive activity to osteoclasts continually cultured at pH6.9 by 24-48 hours.

Conclusion(s): Taken together, the current work indicates that extracellular pH modulates osteoclast fusion and size and may prime cells for subsequent resorptive activity.

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P103

CRISPR/Cas9 mediated dual fluorescent reporter mice to study spatial bone genomics of osteoblasts and osteoclasts in the local in vivo environment

Dilara Yilmaz, Yannick Fischer, Sandra Zimmermann,
Gaonhae Hwang, Ralph Müller, Esther Wehrle
ETH Zurich, Institute for Biomechanics, Zurich, Switzerland

Background/Introduction: To investigate spatial bone genomics *in vivo*, refined mouse models are needed. We have recently CRISPR/Cas9 mediate developed dual fluorescent reporter mice for osteoblasts and osteoclasts, which could allow spatially resolved investigation of bone genomics during adaptation and regeneration of single cells in the local *in vivo* environment (LivE).

Purpose: To characterize our recently developed dual fluorescent reporter mice for osteoblasts and osteoclasts, profound genotypic and phenotypic analyses were performed.

Methods: Osteoblasts were labeled linking GFP with Integrin binding sialoprotein(Ibsp) and osteoclasts were labeled linking mCherry with acid phosphatase type-5 (Acp-5) via CRISPR/Cas genome editing. Unique endogenous signals from osteoblasts were confirmed with Ibsp and GFP whereas specific endogenous signals of osteoclasts were detected through mCherry immunostaining. To validate the specificity of stainings, wild type mice were used as controls.

Results: Specific fluorescent signals for GFP and mCherry were observed around the growth plate and co-localization of endogenous signals with osteoblast-specific GFP and Ibsp, and osteoclast specific mCherry was detected (Fig 1) on femora and vertebrae from 5 and 20 week-old homozygous mice. Wild type controls did not show any endogenous or immunofluorescent signal for GFP and mCherry.

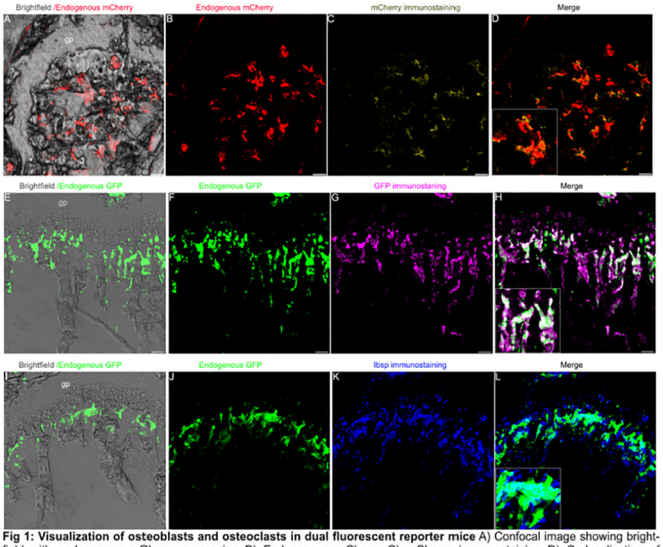

Fig 1: Visualization of osteoblasts and osteoclasts in dual fluorescent reporter mice A) Confocal image showing bright-field with endogenous mCherry expression B) Endogenous mCherry C) mCherry immunostaining D) Co-localization of endogenous mCherry with mCherry immunostainihng E-I) Confocal image showing bright-field with endogenous GFP expression F-J) Endogenous GFP G) GFP immunostaining H) Co-localization of endogenous GFP (green) with GFP immunostaining K) Ibsp immunostaining L) Co-localization of endogenous GFP with osteoblast-specific Ibsp immunostaining. gp:Growth plate.Scale bar:50um

Conclusion(s): The dual-fluorescent reporter mice were generated to identify osteoblasts and osteoclasts fluorescently in mouse bone *in vivo* and the function of fluorescent cells was confirmed with immunohistochemistry. The dual fluorescent reporter mice will be used to study spatially resolved single-cell mechanomics during bone adaptation and regeneration.

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P058

Gastric inhibitory polypeptide (GIP) decreases bone resorptive activity of human osteoclasts in vitro

Morten Steen Svarer Hansen[a], Kent Søe[b], Caroline Gorvin[c,d], Morten Frost[a]
[a]*Odense University Hospital, Department of Endocrinology, Odense, Denmark*
[b]*University of Southern Denmark, Clinical Cell Biology- Pathology Research Unit-Department of Clinical Research, Odense, Denmark*
[c]*University of Birmingham, Institute of Metabolism and Systems Research IMSR and Centre for Diabetes-Endocrinology and Metabolism CEDAM, Birmingham, United Kingdom*
[d]*Universities of Birmingham and Nottingham, Centre for Membrane Proteins and Receptors COMPARE, Birmingham and Nottingham, United Kingdom*

Background/Introduction: GIP is an intestinal hormone that promotes glucose-dependent insulin secretion. Preclinical data indicate a functional GIP receptor on human osteoclasts, and *in vivo* studies has shown GIP acutely decreases biomarkers for bone resorption. However, the direct effects of GIP on human osteoclasts are undetermined.

Purpose: To investigate the effects of GIP on primary human osteoclasts using human osteoclast and osteoclast+osteoblast cell cultures.

Methods: Osteoclasts were differentiated for 10 days from human blood-derived $CD14^+$ monocytes and osteoblasts over 4-6 weeks as outgrowths from human bone specimen. Cells were seeded on bovine bone slices and cultures performed using fetal bovine serum, M-CSF and RANKL (osteoclast monocultures) or M-CSF only (osteoclast+osteoblast co-cultures).

Results: GIP decreased the percentage of eroded bone surface compared to vehicle in osteoclast monocultures (1nM $p<0.01$; 10nM $p<0.01$) and in osteoclast+osteoblast co-cultures (1nM $p<0.05$; 10nM $p<0.05$) but had no effect on osteoblast activity in osteoclast+osteoblast co-cultures (1nM $p=0.94$; 10nM $p=0.08$) (Figure 1). RNAseq documented mRNA expression of GIP receptor in human osteoclasts. For receptor signalling in human osteoclast cultures, we assessed the effects of GIP on cAMP signalling using LANCE assays, on intracellular

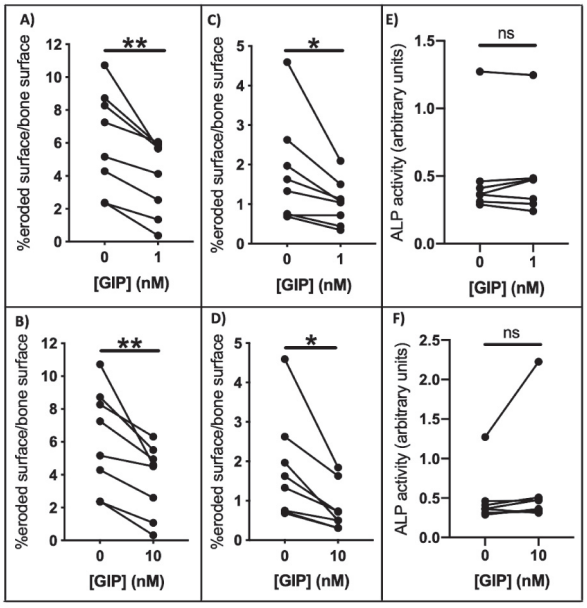

Figure 1: Data points represent 7-8 different experiments each connected with a line. Data are shown as differences in median (six technical replicates pr. experiment) between vehicle and GIP. **A-D)** Osteoclast resorptive activity in osteoclast monoculture (A+B) and osteoclast+osteoblast co-cultures (C+D). Anderson-Darling normality test passed. Paired t-test. *=P<0.05, **=P<0.01 **E-F)** Osteoblast activity assessed by alkaline phosphatase (ALP) activity in osteoclast+osteoblast co-cultures. Wilcoxon matched-pairs signed rank test.

calcium mobilisation Ca^{2+}_i using Fura-2 imaging and phosphorylation of ERK1/2 proteins by Western blot analysis. Osteoclasts treated with 10nM GIP for 30 minutes increased cAMP signalling ($p<0.05$) and Ca^{2+}_i-levels (Emax $p<0.0001$) when compared to vehicle and induced increases in phosphorylated ERK1/2 ($p<0.05$).

Conclusion(s): GIP acts directly on human osteoclasts and has antiresorptive effects *in vitro*.

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Poster Forum

Poster Forum – Clinical

P136

Factors associated with osteoporosis care of men: A real-life study on a nation-wide dataset

Giovanni Adami[a], Davide Gatti[a], Alessandro Giollo[a], Eugenia Bertoldo[a], Ombretta Viapiana[a], Pietro Olivi[b], Angelo Fassio[a], Maurizio Rossini[a]

[a]*University of Verona, Rheumatology Unit, Verona, Italy*

[b]*University of Verona, Orthopedic Unit, Verona, Italy*

Background/Introduction: Male osteoporosis is associated with an important clinical and economic burden worldwide. Notwithstanding that, undertreatment of men with osteoporosis is common. Understanding the factors associated with less osteoporosis care utilization might help define future intervention to improve access of men to osteoporosis care.

Table 1
Clinical and densitometric characteristics of the study population and age-matched cohort of women

	Men (n = 4,902)	Women (n = 9,804)	OR (95% CI) – *p* value
Age (IQR)	67.0 (56.0–75.0)	67.0 (56.0–75.0)	NS
BMI (±SD)	25.31 (±4.91)	24.07 (±4.85)	**<0.0001**
Lumbar spine T-score (IQR)	-2.50 (-3.00–1.40)	-2.60 (-3.20–1.93)	**<0.0001**
Osteoporosis at lumbar spine (%)	2,601 (53.1%)	5,948 (60.7%)	**0.733 (0.684–0.785)**
Femoral neck T-score (±SD)	-2.10 (-2.50–1.44))	-2.20 (-2.80–1.70)	**<0.0001**
Osteoporosis at femoral neck (%)	1,412 (28.8%)	3,936 (40.1%)	**0.603 (0.560–0.649)**
% 10-year risk of fracture (±SD)	22.77 (±21.05)	20.26 (±4.85)	**<0.0001**
Family history of fragility fracture (%)	891 (18.2%)	2,379 (24.3%)	**0.693 (0.636–0.756)**
Secondary osteoporosis (%)	2,415 (49.3%)	3,092 (31.5%)	**2.108 (1.965–2.262)**
Glucocorticoids ≥5 mg/day >3 months (%)	768 (17.4%)	694 (7.7%)	**2.573 (2.274–2.832)**
Glucocorticoids ≥5 mg/day ≥3 months <12 months (%)	74 (2.0%)	72 (0.9%)	**2.357 (1.700–3.267)**
Glucocorticoids ≥5 mg/day ≥12 months (%)	119 (3.2%)	138 (1.6%)	**1.977 (1.543–2.534)**
Adjuvant hormonal therapy for breast or prostate cancer (%)	495 (12.0%)	766 (8.4%)	**1.482 (1.315–1.670)**
Comorbidities (%)	1,778 (36.3%)	2,225 (22.7%)	**1.939 (1.799–2.090)**
Rheumatoid arthritis (%)	303 (8.8%)	511 (6.3%)	**1.439 (1.241–1.668)**
Psoriatic arthritis (%)	59 (1.9%)	103 (1.3)	**1.390 (1.006–1.919)**
Systemic lupus erythematosus (%)	22 (0.7%)	77 (1.0%)	0.693 (0.431–1.115)
Systemic sclerosis (%)	9 (0.3%)	60 (0.8%)	**0.364 (0.180–0.734)**
Other rheumatic diseases (%)	173 (5.2%)	310 (3.9%)	**1.354 (1.119–1.638)**
Inflammatory bowel diseases (%)	144 (2.7%)	126 (1.6%)	**2.773 (2.175–3.534)**
Chronic obstructive pulmonary disease (%)	281 (8.3%)	277 (3.5%)	**2.461 (2.074–2.920)**
Diabetes (%)	441 (12.4%)	478 (5.9%)	**2.238 (1.954–2.564)**
Neurological diseases (%)	236 (7.0%)	260 (3.3%)	**2.202 (1.837–2.639)**
HIV infection (%)	110 (3.4%)	23 (0.3%)	**11.603 (7.389–18.221)**
Vertebral or hip fractures (%)	1,434 (29.3%)	2,130 (21.7%)	**1.490 (1.378–1.611)**
Non-vertebral, non-hip fractures (%)	534 (10.9%)	1,477 (15.1%)	**0.689 (0.620–0.766)**

Purpose: The aim of the study was to describe the factors associated with osteoporosis care in men.

Methods: We conducted a retrospective analysis of a nation-wide cohort (DeFRACalc79 database). DeFRACalc79 is a tool that estimates the fracture risk considering clinical and densitometric risk factors, including the presence of prior hip or vertebral and non-vertebral or non-hip fractures. We compared the clinical characteristics of male individuals with an age matched cohort of women. Propensity score generation with 2:1 matching for female and male patients was performed matching the cohorts for age, generating propensity estimates with a logistic regression model.

Results: We analyzed a sample of 4,902 men at high risk of osteoporosis. We found that the factors associated to osteoporosis care utilization in men were: the presence of comorbidities (OR 1.939, 95% CI 1.799-2.090), adjuvant hormonal therapy for prostate cancer (OR 1.482, 95% CI 1.315-1.670), the presence of vertebral or hip fractures (OR 1.490, 95% CI 1.378-1.611) and glucocorticoid treatment (OR 2.573, 95% CI 2.274-2.832) (**table 1**)

2352-1872/$ – see front matter

Conclusion(s): We found that men accessed osteoporosis care with more severe osteoporosis and/or with a diagnosis of secondary osteoporosis. Male osteoporosis remains largely underdiagnosed with a dramatic latency in osteoporosis care utilization compared to women.

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P122

Developing a service for the incidental identification of osteoporotic vertebral fractures and the secondary fragility fracture prevention using artificial intelligence

Eleni Kariki[a,b], Paul Bromiley[b], Jonathan Roberts[c], Jon Parkinson[b], Stan Thompson[d,e], Timothy Cootes[b], Shawn Luetchens[f]
[a]*Manchester University NHS Foundation Trust-Manchester Royal Infirmary, Department of Clinical Radiology, Manchester, United Kingdom*
[b]*University of Manchester, Faculty of Biology Medicine and Health- Division of Informatics Imaging & Data Sciences, Manchester, United Kingdom*
[c]*Optasia Medical Ltd., Research & Development- Machine Learning Development, Manchester, United Kingdom*
[d]*University of Manchester, Patient and Public Involvement representative, Manchester, United Kingdom*
[e]*Royal Osteoporosis Society, Central Manchester Patient Support Group, Manchester, United Kingdom*
[f]*Optasia Medical Ltd., Chief Executive Officer, London, United Kingdom*

Background/Introduction: Osteoporosis is a chronic progressive disease. It affects over 200 million people worldwide. Osteoporosis presents with low impact-fragility-fractures (oFFs), for example of the vertebrae, hip, wrist. There are 9 million new oFFs annually. Osteoporotic vertebral fractures (oVFs) occur earlier in the course of the disease compared to other oFFs, rarely come to clinical attention and increase the risk of secondary oFFs. However, only 13-46% of the patients with oVFs are identified, even less are appropriately managed. People with unmanaged osteoporosis eventually suffer with a range of physical and mental complaints that make their everyday lives a struggle. We can prevent the grave impact osteoporosis has on the person, our health and social care systems and our societies.

Purpose: Effectively identify people with oVFs and provide adequate care to prevent further oFFs.

Methods: We developed an artificial intelligence software for the incidental identification of oVFs on Computed Tomography-CT images. We used this tool to design a service that automates the referral of people with undiagnosed osteoporosis for bone-health management. The tool retrieves imaging examinations from Picture Archiving and Communication Systems (PACS). The machine learning models use 3D image-data to form a single 2D mid-sagittal image of the spine. The tool identify and describe the findings following the current guidelines, producing accurate, informative and actionable radiology reports. The service includes a patient management application helping with patient prioritization and referral for appropriate bone-health management.

Results: Our service has been piloted in more than 10,000 patients 50 years and older. We identified oVFs in 20.6% of the patients, 67% not previously reported. Our service referred more than 2,000 patients for appropriate bone-health management. In total, 94.8% of the patients with oVFs would have otherwise remained unmanaged.

Conclusion(s): Artificial intelligence can be used to provide holistic healthcare to people with osteoporosis, efficiently synchronizing primary and secondary care.

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P154

Zoledronic acid effectively inhibited periprosthetic bone loss in patients with osteoporosis after cementless total hip arthroplasty

Yue Ding, Changchuan Li, Yuxi Huang, Sipeng Lin, Zhongcan Zheng
SYSU, Orthopedics, Guangzhou, China

Background/Introduction: Total hip arthroplasty (THA) is the most effective therapy for end-stage hip disease. It was reported that low bone mineral density (BMD) contributed to advanced implant migration in the early stage after THA, accompanied with worse initial stability. However, it remains unclear whether patients with osteopenia or osteoporosis would experience accelerated postoperative periprosthetic bone loss, and whether zoledronate acid (ZOL) could reduce periprosthetic bone loss after THA.

Purpose: To investigate the influence of preoperative osteopenia/osteoporosis on periprosthetic bone loss after cementless THA, and the efficiency of ZOL in periprosthetic bone preservation.

Methods: This multicenter, prospective cohort study was conducted in four centers between April 2015 and September 2019. Patients were assigned to Normal BMD, Osteopenia, and Osteoporosis groups. Patients with osteopenia received daily oral calcium (600 mg/d) and vitamin D (0.5 μg/d), while patients in the Osteoporosis group received additional ZOL annually (5 mg/year). Periprosthetic bone mineral density (BMD) in seven Gruen zones,and BMD in hip and spine were measured within 7 days, 12 months, 24months postoperation and annually thereafter.

Results: A total of 181 patients were enrolled, while 10 patients who completed the second year follow-up were involved in the statistical analysis. The average duration of follow-up was 2.4 years. There was significant decrease of mean BMD in total Gruen zone1 (−14.31%) and Gruen zone 7 (−16.86%) in patients with osteopenia during the second postoperative year. Patients in the Osteoporosis group experienced a marked increase in BMD in Gruen zone 1 (+13%) at the second postoperative year,when compared with the Normal BMD group and the Osteopenia group. However, the difference was not statistically significant.

Conclusion(s): Patients with osteopenia are prone to higher bone loss in the proximal femur after cementless THA. ZOL, not solely calcium and vitamin D, could prevent the accelerated periprosthetic bone loss after THA in patients with osteopenia and osteoporosis.

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P169

Adenosine Deaminase 2 Deficiency: Its pleiotropic manifestations may also affect bone metabolism? A case study on two homozygous female twins

Erika Marin[a], Iacopo Chiodini[b], Luca Persani[b], Alberto Falchetti[c], Silvia Vai[d]
[a]*Istituto Auxologico Italiano, Experimental Laboratory for Children Bone Metabolism Research, Cusano Milanino, Italy*
[b]*Istituto Auxologico Italiano, Unit for Bone Metabolism Diseases and Diabetes, Milano, Italy*
[c]*Istituto Auxologico Italiano, Unit for Bone Metabolism Diseases and Diabetes, Piancavallo, Italy*
[d]*Istituto Auxologico Italiano, Experimental Laboratory for Children Bone Metabolism Research, Milano, Italy*

Background/Introduction: Adenosine Deaminase 2 Deficiency (DADA2) is a rare immune system disease caused by the absence of enzymatic activity. Generally, it manifests at infancy/early childhood, associated with pleiotropic manifestations including stroke, systemic

vasculitis, hematologic compromise, and immunodeficiency. Bone marrow transplantation (BMT) may be required for severe cases. No Information on bone health is available in DADA2.

Purpose: We present a case study of two 27 years-old DADA2 female twins, T1 and T2, manifesting systemic inflammation and vasculitis. T1 suffered 2 ischemic strokes (2002, 2008), and with hypothyroidism (2016). T2, with hypothyroidism, had 2 ischemic strokes (1996, 1997) and T-LGL like expansion for which underwent BMT in 2018. In 2018, she had also the diagnosis of POF, treated with HRT. DNA test revealed CERC1/ADA2 gene c.T203C:pL68P germline mutation in both.

Methods: T1-T2 bone health was monitored by DXA-BMD scans at lumbar spine (LS), total hip/femoral neck (TH/FN) and total body (TB) [g/cm2, and standard deviation (SD) Z-score (Zs)], LS X-rays, and bone metabolism parameters.

Results: T1 has low BMD (DXA-LS = 0.775, Zs -2.4) and vitamin D insufficiency, supplemented. T2 had low BMD since 2013 (FN=0.653, Zs -2.4), worsened after BMT (LS=0.687, Zs -3.5, TH=0.608, Zs -3.1) in 2018. Infusions with i.v. 100 mg neridronate, together with vitamin D supplementation, resulted in DXA-LS LS=0.908, Zs -1.5, and -TH=0.718, Zs -2.1 in 2020.

Conclusion(s): T1-T2 low BMD is due to failure to achieve adequate Peak Bone Mass, but whether this directly links to DADA2 and/or environmental factors, concomitance polytherapy (acyclovir and fluconazole) is uncertain. POF and BMT may play a major role in low BMD in T2, but T1 not suffered for any of these. We cannot rule out if epigenetic mechanisms or modifying genes contributed to low BMD. These preliminary data suggest that in DADA2 patients adequate/appropriate bone health status should be also monitored.

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P030

The importance of retaining physical functions to prevent skeletal-related events in multiple myeloma patients with bone disease

Hirokazu Miki[a], Nakamura Shingen[b], Masafumi Nakamura[c], Makiko Mizuguchi[c], Ryohei Sumitani[c], Masahiro Oura[c], Kimiko Sogabe[c], Mamiko Takahashi[c], Tomoko Maruhashi[c], Harada Takeshi[c], Shiro Fujii[c], Kumiko Kagawa[c], Hirofumi Hamano[d], Masateru Kondo[e], Naoto Okada[e], Yoshimi Bando[f], Itsuro Endo[c], Masahiro Abe[c]

[a]*Tokushima University Hospital, Division of Transfusion Medicine and Cell Therapy, Tokushima, Japan*

[b]*Tokushima University Graduate School of Biomedical Sciences, Department of Community Medicine and Medical Science, Tokushima, Japan*

[c]*Tokushima University Graduate School, Department of Hematology-Endocrinology and Metabolism- Institute of Biomedical Sciences, Tokushima, Japan*

[d]*Tokushima University Graduate School, Department of Clinical Pharmacology and Therapeutics- Institute of Biomedical Sciences, Tokushima, Japan*

[e]*Tokushima University Hospital, Department of Pharmacy, Tokushima, Japan*

[f]*Tokushima University Hospital, Division of Pathology, Tokushima, Japan*

Background/Introduction: Bone destruction is a debilitating clinical event in patients with multiple myeloma (MM). Denosumab or zoledronic acid are regarded as a standard of care for MM bone disease. MM tumor progression is generally accepted to be among major causative factors for skeletal-related events (SRE) occurrence in MM patients.

Purpose: To make efficacious care to prevent SRE occurrence, we analyzed to identify baseline conditions and triggering factors for SRE occurrence in patients with MM on denosumab.

Methods: We retrospectively analyzed MM patients who received denosumab from 2012 to 2020 in Tokushima University Hospital.

Results: MM patients (37 males and 38 females) with a median age of 69 years old (range 44-88) were treated with denosumab. At the median follow-up of 17 months (IQR 1-86), 11 patients experienced SRE. SRE occurred in 6 out of 52 newly diagnosed patients, 5 out of 23 relapsed/refractory patients. Univariate logistic regression analysis revealed that combination with AL amyloidosis (odds ratio 23.93, p=0.009) and PS at the baseline with ECOG 3 or 4 (odds ratio 4.50, p=0.037) were significant risk factors for SRE. In multivariate logistic regression analysis, combination with AL amyloidosis was an independent risk factor for SRE (odds ratio 14.62, p=0.035). Bone fractures occurred by falling down in 3 out of 4 cases with AL amyloidosis. Balance loss and falling down due to orthostatic hypotension and/or muscle weakness were observed in 8 out of 11 cases with SRE at the time of fractures.

Conclusion(s): To prevent progression of bone disease in MM, implementation of novel anti-MM treatment modalities to exert deeper and more durable response is expected; nonetheless, balance loss and falling down due to orthostatic hypotension and/or muscle weakness appear to be triggering factors for bone fracture especially in frail MM patients and those with AL amyloidosis, indicating the importance of retaining physical functions to prevent SRE.

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P146

Pathogenesis of osteoporosis in patients with systemic or cutaneous mastozytosis

Christof Wilke[a], Martina Blaschke[a], Annette Lamersdorf[b], Christina Heppner[b], Michael Metz[c], Hans-Peter Horny[d], Undine Lippert[e], Heide Siggelkow[b]

[a]*University Medical Center Göttingen, Clinic of Gastroenterology-Gastrointestinal Oncology and Endocrinology, Goettingen, Germany*

[b]*MVZ endokrinologikum Göttingen, MVZ endokrinologikum Göttingen, Göttingen, Germany*

[c]*Practice for hematology and oncology, Practice for hematology and oncology, Göttingen, Germany*

[d]*Ludwig-Maximilian-University, Institute of Pathology, München, Germany*

[e]*University Medical Center Göttingen, Department of Dermatology-Venereology and Allergology, Göttingen, Germany*

Background/Introduction: Mastocytosis (MC) is a complex disease characterized by an aggregation of mast cells in the skin (cutaneous MC; CM) or in other organs (systemic MC; SM). In SM mast cells often infiltrate bone marrow, which can cause osteoporosis by activating osteoclasts and thereby decreasing bone mineral density (BMD). Consequently, these patients suffer from low trauma fractures, especially at the spine. However, osteoporosis is also diagnosed in patients with CM. A bone biopsy with verifiable mast cell infiltrates is essential to differentiated between CM and SM. High tryptase level, *cKit*-mutation or skin lesions are further diagnostic criteria for MC.

Purpose: Identifying differences concerning bone affection between SM and CM

Methods: In our retrospective one-center study, we analyzed clinical data (e.g. age, gender, skin lesions, allergy, back pain, bone mineral density, trabecular bone score (TBS), fractures, tryptase, bone turnover markers, bone marrow mast cell infiltration and *cKIT* mutation) from 106 patients, who were diagnosed for mastocytosis between 2006 and 2019.

Results: Selected data are summarized in table 1. Tryptase did not correlate to fractures, BMD or TBS.

Table 1
Selected Parameters analyzed in MC patients (mean age 57±13 years)

	CM (n=9; m=1;w=8)	SM (n=44; m=10;w=34)	ISM suspected*(n=32; m=7;w=25)
BMD:Osteopenic/ Osteoporotic % (n)	38 (3)/38 (3)	49 (18)/41 (15)	36 (9)/44 (11)
Fractures: spin/ peripheral (n/patient)	0.28/0.28	1.3/0.4	1.5/0.4
Tryptase (µg/L)	12.84±6.80 (9)	59.14±50.13 (39)	17.33±6.66 (28)
Bone alkaline phosphate (µg/L)	21.91±12.34 (9)	26.79±14.83 (43)	26.81±12.49 (30)
Deoxypyridinoline (µg/L)	20.47±8.83 (8)	22.98±9.45 (43)	25.24±12.09 (28)
Back pain (scores 0-10)	2.06±2.54 (8)	4.32±2.47 (38)	3.42±2.98 (26)

* elevated tryptase levels; evidence for MC e.g.skin lesions, allergy, low trauma fractures

Conclusion(s): The higher incidence of osteopenia and osteoporosis in SM patients is associated with higher tryptase levels, more back pain, a higher number of individual fractures and increased bone turnover markers.

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Poster Forum – Basic/Translational

P032
Differential effects of zoledronic acid and oestrogen on anti-cancer immunity

Chris George, Diane Lefley, Victor Canuas-Landero, Claudia Tulotta, Hannah Corness, Caroline Wilson, Munitta Muthana, Penelope Ottewell
University of Sheffield, Oncology and Metabolism, Sheffield, United Kingdom

Background/Introduction: Late stage breast cancer predominantly recurs in bone. Neoadjuvant Zoledronic Acid (ZOL) combined with standard of care has shown to reduce bone metastases in pre- and post- menopausal women and have life prolonging benefits in post-menopausal women. However, the same treatment results in shorter life expectancy and increased tumour recurrence outside of bone in pre-menopausal women. Evidence suggests that ZOL and oestrogen affect bone homeostasis and immune cells.

Purpose: We hypothesise that oestrogen may inhibit anti-tumour activities of ZOL in pre-menopausal women through immune regulatory pathways.

Methods: Ovariectomy followed by 10 pM/L, 84 pM/L and 300pM/L oestradiol were used to model pre, peri and post-menopausal conditions in 12-week old mice. Metastases were induced by intracardiac or intra-ductal injection of mouse mammary cancer E0771 and 4T1 cells before treatment with 100ug/kg/week ZOL or control. Effects on tumours, bone, immune cells and hormones were assessed by IVIS imaging, uCT, histomorphology, flow cytometry, NanoString and ELISA.

Results: Oestradiol administration to ovariectomised mice (n=60) reliably recreated pre- peri and post- menopausal serum concentrations observed in humans. IVIS analysis demonstrated increased non bone metastasis in pre-menopausal mice following ZOL treatment, mirroring clinical findings. ZOL increased bone density in post-menopausal mice (P=0.0034), and decreased activity of osteoblasts (P1NP) (P=0.001) and osteoclasts (TRACP) (P=<0.001) under pre-post and peri-menopausal conditions. In contrast, pre-menopausal concentrations of oestradiol significantly increased trabecular bone volume (P=0.06) and increased osteoblast activity in the presence of ZOL (P=0.04). Data from flow cytometry and immunohistochemistry suggest that ZOL and oestradiol also exert deferential effects on immune cell populations, with oestrogen increasing M2 macrophage populations, and Zol increasing M1 (P=0.0374) populations in the bone.

Conclusion(s): Oestradiol impedes the ability of ZOL to inhibit osteoclast activity and exerts alterations to the tumour microenvironment leading to reduced anti-tumour effects observed under pre-menopausal conditions.

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P064
Rat perichondrium transplanted to articular cartilage defects forms articular-like, hyaline cartilage

Zelong Dou[a], Daniel Muder[b], Marta Baroncelli[a], Ameya Bendre[a], Alexandra Gkourogianni[a], Lars Ottosson[a], Torbjörn Vedung[b], Ola Nilsson[a]
[a]*Karolinska Institutet, Women's and Children's Health, Stockholm, Sweden*
[b]*Uppsala University, Surgical Sciences, Uppsala, Sweden*

Background/Introduction: Reconstruction of articular surfaces destroyed by infection or trauma is hampered by lack of suitable graft tissues. Perichondrium autotransplants have been used for this purpose. However, the role of the transplanted perichondrium in the healing of resurfaced joints have not been investigated.

Purpose: To investigate the contribution of perichondrium transplants to healing and chondrogenesis in articular cartilage defects.

Methods: Perichondrial and periosteal tissues were harvested from 6-week-old rats hemizygous for a ubiquitously expressed enhanced green fluorescent protein (EGFP) transgene and transplanted into full-thickness articular cartilage defects at the distal femoral trochlear groove in wild-type littermates. As an additional control, cartilage defects were left without a transplant. Distal femurs were collected 3, 14, 56, 112 days after surgery. Ethical No. N248/15.

Results: Transplanted cells and their progenies were readily detected in the defects of perichondrium and periosteum transplanted animals. Perichondrium transplants differentiated into a hyaline cartilage that expanded and filled out the defects with *Col2a1*-positive chondrocytes ($P<0.01$ vs periosteum and no transplant control) and a matrix rich in proteoglycans (Fig 1. $P=0.0022$ vs periosteum and no transplant control). In contrast, periosteum transplants exhibited transient chondrogenic differentiation, but remained *Col1* positive, and were continuously thinning and lost *Col2a1* expression and proteoglycan content.

Conclusion(s): Perichondrium, but not periosteum, transplanted to articular cartilage defects developed into hyaline cartilage that integrated with the subchondral bone and was maintained for at least 112 days. The findings suggest that perichondrium is a suitable tissue for repair and engineering of articular cartilage.

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P171
The combination of hemodynamic alterations and endothelial dysfunction accelerates arterial media calcification in warfarin administered rats

Geoffrey Van Den Bergh, Astrid Van den Branden, Patrick D'Haese, Anja Verhulst
University of Antwerp, Biomedical Sciences, Wilrijk, Belgium

Background/Introduction: Arterial media calcification is an independent risk factor for cardiovascular events. The calcification process is complex and controlled active cell process. This

encompasses an integral role for vascular smooth muscle cells by mediating osteochondrogenic transdifferentiation and the generation of nucleation sites for the deposit of crystals. Increasing evidence suggests a significant role for endothelial cells in the development of arterial media calcification.

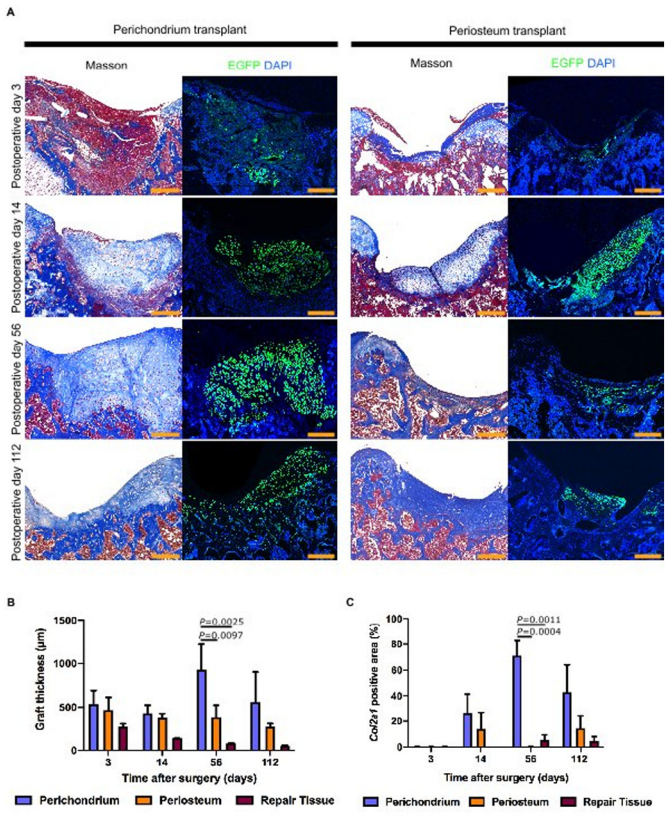

Fig1. Histology and transplanted cells tracing of cartilage defects repaired with perichondrium or periosteum transplants

Purpose: In particular, endothelial dysfunction, characterized by an impaired nitric oxide bioavailability, and its contribution to the pathophysiology of arterial media calcification was explored in this study.

Methods: Male rats (n=40) were randomly assigned over 4 different groups. The first group (n = 5) received standard chow. The second group (n=5) were given L-NAME (0,5 g/L/body weight) in addition to standard chow. The third group (n=15) and fourth group (n=15) received a warfarin-supplemented diet to induce calcification and the latter co-administered L-NAME. Prior to sacrifice, hemodynamic parameters were measured: non-invasive measurement of the blood pressure and aortic distensibility. Animals were sacrificed after 6 weeks. Arterial media calcification was quantified via atomic absorption spectrometry. *Ex vivo* vascular reactivity was assessed via organ baths.

Results: Warfarin intake was similar for both groups receiving the warfarin diet ($p>0.05$). Systolic and diastolic blood pressure were significantly elevated ($p<0.05$), while aortic distensibility was significantly lower ($p<0.05$) in groups which received L-NAME. *Ex vivo* aortic reactivity on isolated segments established the presence of endothelial dysfunction ($p<0.01$). Aortic calcium was significantly increased compared to controls in both groups receiving warfarin ($p<0.01$). Moreover, the group receiving both warfarin and L-NAME did have significantly elevated bulk calcium measurements compared to the warfarin group without L-NAME ($p<0.05$).

Conclusion(s): To the best of our knowledge, this is the first *in vivo* study which describes a combined role for altered hemodynamics and endothelial dysfunction in the pathophysiology of arterial media calcification.

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P202

Introducing and restoring Craniosynostosis-related gene mutations using prime-editing

Max Gijsbertsen[a], Joël Kraaijenbrink[a], Moustapha Kassem[b], John van der Oost[c], Irene Mathijssen[d], Hans van Leeuwen[a], Jeroen van de Peppel[a]
[a]*Erasmus MC, Internal Medicine, Rotterdam, Netherlands*
[b]*Odense University Hospital, Endocrinology and Metabolism, Odense, Denmark*
[c]*Wageningen University and Research, Laboratory of Microbiology, Wageningen, Netherlands*
[d]*Erasmus MC, Plastic and Reconstructive Surgery, Rotterdam, Netherlands*

Background/Introduction: Craniosynostosis is a rare congenital condition where one or more calvarial sutures prematurely fuse together, resulting in aberrant skull and brain growth often accompanied with craniofacial deformities and intellectual retardation. Currently, highly invasive surgeries are the common course to relieve cranial pressure. Due to the multigeneity of Craniosynostosis, the condition lacks reliable cell model systems and establishing cell models based on the variety of causative mutations is extensive and difficult. Prime-editing is a new variant of CRISPR/Cas9 capable of inducing specific mutations in target DNA with high accuracy.

Purpose: Using Prime-editing we aim to incorporate Craniosynostosis-related gene mutations in a cell line, in order to ultimately generate in vitro disease models of monogenic bone diseases that can be utilized for further research. Additionally, with the generation of patient-specific induced Pluripotent Stem Cells (iPSC), we aim to restore the causative mutation from the patient's genome using the same technique.

Methods: Custom designed Prime-editing guide RNAs (pegRNA) targeting FGFR2 are generated, and together with the Prime-editor Cas9, transfected into TERT immortalized MSCs. After 5 days of editing genomic DNA (gDNA) is isolated, and analyzed by Edit-specific PCR, restriction enzyme digestion analysis, and Sanger Sequencing.

Results: A 3 base-pair insertion in HEK3 was successfully incorporated in HEK293FT and MSC-TERT cells. Custom pegRNAs targeting FGFR2 were successfully generated, but required optimization in order to be functionally utilized in prime-editing. Patient specific iPSCs (Crouzon Syndrome) were successfully generated illustrated by stem cell marker expression (OCT4, TRA-1-81, NANOG, and SSEA4), and were shown to differentiate into each of the three germ layers.

Conclusion(s): Prime-editing can be effectively used to introduce mutations in both HEK239FT and MSC-TERT cells. Functionality of pegRNA transcripts heavily rely on the secondary structures that are formed. Patient specific iPSCs are successfully generated.

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P034

Site-specific alterations in murine cortical bone porosity: Enlarged osteocyte lacunae and dense vascular channels in tibia inflicted with osteotropic tumor cells

Haniyeh Hemmatian[a], Stefanie Conrad[b], Giulia Furesi[b], Martina Rauner[b], Björn Busse[a], Katharina Jähn-Rickert[a]
[a]*University Medical Center Hamburg-Eppendorf, Department of Osteology and Biomechanics, Hamburg, Germany*
[b]*Technische Universität Dresden, Department of Medicine III and Center for Healthy Aging, Dresden, Germany*

Background/Introduction: Bone metastases are debilitating consequences of breast and prostate cancers, producing either osteolytic, osteosclerotic, or mixed lesions, through activation of a 'vicious cycle' impairing the homeostatic bone remodeling. During metastasis formation, increased blood vessel invasion supports

tumor growth, but also delivers progenitor cells for osteoclasts and osteoblasts. Mechanosensitive osteocytes are central regulators of bone remodeling by osteoclasts and osteoblasts, but also actively remodel their perilacunar matrix, playing a key role in maintaining mineral homeostasis. However, a paucity of data exists on tumor-associated osteocyte interactions. If any, these variations in the lacunar morphology may cause changes in the osteocyte mechano-environment, subsequently affecting bone mechanotransduction resulting in an impaired bone remodeling through vicious cycle.

Purpose: This study aimed to quantify the effect of osteotropic cancer on cortical bone microporosities, including the osteocyte lacunar network and vascular canals.

Methods: The structural heterogeneity of bone microporosity was evaluated in the proximal tibial cortex, 15 days after intratibial injection of metastatic breast EO771-Luc ($1x10^5$) and prostate RM1-LNCX ($5x10^3$) cancer cells using high resolution micro-computed tomography (μCT) and confocal microscopy following ethical approval (TVV 18/2015, 3/2017).

Results: Confocal imaging highlighted direct contact between tumor cells and osteocytes. High-resolution μCT analysis revealed 12% (p-value=0.01) and 10% (p-value=0.04) increase in lacunar volume after intratibial EO771-Luc breast and RM1-LNCX prostate cancer cell injection, respectively. The 3D reconstruction of lacunar and vascular canals spatial distribution showed regional accumulation of large lacunae and vascular canals adjacent to osteosclerotic lesions and the absence of large lacunae and vascular canals near osteolytic lesions.

Conclusion(s): These findings suggest that the presence of tumor cells in the bone microenvironment affects osteocyte lacunar characteristics and cortical bone blood vessel structure. The characterization of the interaction between osteocytes and tumor cells may provide new mechanistic insights into pathological changes in bone tissue with metastasis formation.

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Poster

P001

Characterisation of sarcopenia via DXA measured muscle/fat mass parameters and uc-dpMGP serum levels

Natascha Schweighofer[a], Moritz Strasser[a], Christoph Haudum[a], Albrecht Schmidt[b], Thomas Pieber[a], Barbara Obermayer-Pietsch[c]

[a]*Medical University Graz, Internal Medicine - Division of Endocrinology and Diabetology- CBmed Graz, Graz, Austria*

[b]*Medical University Graz, Internal Medicine - Division of Cardiology, Graz, Austria*

[c]*Medical University Graz, Internal Medicine- Division of Endocrinology and Diabetology, Graz, Austria*

Background/Introduction: Sarcopenia is a degenerative loss of skeletal muscle mass, quality, and strength. Increased risk of falls and fractures lead to mobility disorders, decreased quality of life, loss of independence and increased mortality.

Since matrix-GLA protein (MGP) is associated with bone parameters, we investigated whether uncarboxylated, dephosphorylated MGP (uc-dpMGP) is associated with sarcopenia and muscle/fat mass parameters.

Purpose: Since matrix-GLA protein (MGP) is associated with bone parameters, we investigated whether uncarboxylated, dephosphorylated MGP (uc-dpMGP) is associated with sarcopenia and muscle/fat mass parameters.

Methods: We analysed data from the BioPersMed cohort (n=966, 531 females, 435 males, mean age 58 +/- 9 years), a prospective cohort of asymptomatic subjects at cardiovascular risk. Uc-dpMGP was measured via IDS-iSYS Ina*K*tif MGP assay (Immunodiagnostic Systems, UK). Muscle/fat mass parameters were determined by Lunar iDXA (GE Healthcare GmbH, Austria): Appendicular lean mass (ALM); lean mass index (LMI); fat mass index (FMI); lower extremity skeletal muscle mass (LESM); total skeletal mass (TSM); total skeletal mass index (TSMI); $ALMI^{BMI}$:ALMI/BMI. Hand grip strength (HGS) was measured via Jamar hydraulic hand dynamometer (Patterson Medical Ltd., UK).

Results: Sarcopenia was specifically defined via HGS at the weaker hand; SMI (skeletal muscle mass index); LESMI (lower extremity skeletal muscle mass); AMMI (appendicular skeletal muscle mass index; ASM (appendicular skeletal muscle mass); ALMI (appendicular lean mass index). The number of persons with sarcopenia varied between 29 and 224 according to definition parameters.

ALM (p=0.004), LMI (p=0.004), FMI (p<0.001), LESM (p=0.004), TSM (p=0.014) and TSMI (p=0.004) correlated positively and ALMI_BMI (p<0.001) negatively with uc-dpMGP serum levels. Uc-dpMGP levels were significantly higher in persons with sarcopenia according to SMI (p=<0.001), HGS (p=0.008), AMMI (p=0.011), ASM (p=0.001) and all parameters (p<0.001).

Conclusion(s): Uc-dpMGP serum levels are potential biomarkers for the characterisation of sarcopenia. However, their associations are depending on the indices of sarcopenia definition and will be further investigated.

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P003

Association of circulating biomarkers with osteoporosis and sarcopenia in community-dwelling postmenopausal women in China

Qun Cheng

Huadong Hospital affiliated to Fudan University, Department of Osteoporosis and Bone Disease, Shanghai, China

Background/Introduction: Sarcopenia and osteoporosis are major health issues in aging populations. There are still lack of good biomarkers for osteoporosis and sarcopenia.

Purpose: The purpose of this study was to investigate the correlations of circulating biomarkers with bone mass, muscle mass and strength, and identify the appropriate biomarkers predicting bone loss and muscle disfunction in postmenopausal women.

Methods: A cross-sectional study on healthy community-dwelling women aged 20-90 was performed to assess the relationship of myokines, sex hormone and bone markers with bone mass, muscle mass and strength using t-test, ANOVA, Pearson's, and multivariate linear regression.

Results: Serum DHEA was positively correlated with muscle strength and bone mass of lumbar spine and hip, and serum oxytocin was positively correlated with muscle mass and bone mass of total hip and femoral neck, however, serum follistatin was negatively correlated with muscle mass, and myostatin was positively correlated with fat mass. After adjusted by age and BMI, serum DHEA and oxytocin were still positively associated with bone mass, muscle mass, and strength.

Conclusion(s): Circulating DHEA and follistatin may be biomarkers of sarcopenia, and circulating oxytocin may be a biomarker of osteosarcopenia in the postmenopausal women.

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P004

MicroRNAs in diabetic bone disease: A systematic review

Ankita Duseja[a], Lisa Dowling[a], Tatiane Vilaca[a], Patryk Zarecki[a], Richard Eastell[a], Matthias Hackl[b]

[a]*University of Sheffield, Oncology & Metabolism, Sheffield, United Kingdom*

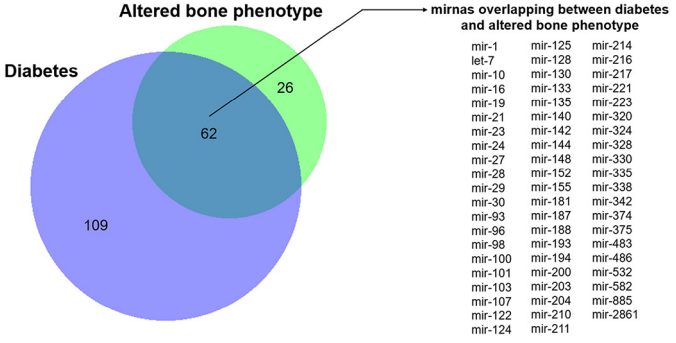
[b]*TAmiRNA GmbH, Biomarker Development, Vienna, Austria*

Background/Introduction: The increase in fracture risk in diabetes is not explained by changes in bone mineral density. Therefore novel biomarkers such as microRNAs (miRNAs/mirnas) are needed to improve the accuracy of fracture risk prediction in diabetes.

Purpose: Differentially expressed miRNA profiles have been identified in patients with diabetes and altered bone phenotype individually. Thus, we performed a systematic review of the literature to identify those differentially expressed miRNAs overlapping between diabetes and altered bone phenotype. The aim of this systematic review is to identify a mirna profile for diabetic bone disease that can be used alongside standard bone density measurement tools for better fracture risk prediction in diabetic patients.

Methods: The search was performed in two parts using PubMed, Ovid MEDLINE and Web of Science. Search part 1 identified all studies that compared miRNA expressions between diabetic patients and non-diabetic controls. Search part 2 identified all studies that compared miRNA expressions between patients with altered and normal bone phenotype. miRNA expression levels of $p<0.05$ were included in this analysis. mirnas overlapping between the two searches were selected to form a preliminary mirna panel for diabetic bone disease. The protocol for this systematic review was registered in PROSPERO (CRD42020212451).

Results: 62 mirnas were found to overlap between patients with diabetes and altered bone phenotype. These mirnas are known to play key roles in glucose metabolism and bone remodelling.



Conclusion(s): Our results identified a preliminary mirna panel comprising of 62 mirnas differentially expressed in both diabetes and altered bone phenotype. This mirna panel has the potential to be used alongside bone density measurement tools for accurate fracture risk prediction and help better understand diabetic bone disease.

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P005

Bisphosphonates may induce flu-like syndrome throw the activation of the interferon-I-regulated pathway

Mikhail Kostik
Saint-Petersburg State Pediatric Medical University- Saint-Petersburg, Hospital Pediatry, Saint-Petersburg, Russia C.I.S.

Background/Introduction: Bisphosphonates (BF) are being used in million patients with osteoporosis and different bone metabolic diseases. The main adverse event of BF is a flu-like syndrome, which is caused by the excessive cytokine release, exposed by BF. The precise mechanism of flu-like syndrome is unknown. Nowadays interferon-I- regulated pathway is considered an important pathogenesis component of different immunological conditions while data on its' role in chronic nonbacterial osteomyelitis (CNO) are scarce.

Purpose: The aim of our study was to evaluate activity of Interferon-I mediated pathway in CNO treated with BF.

Methods: In the prospective study were included children with chronic non-bacterial osteomyelitis CNO, requiring BF treatment (n=9). Activity of Interferon-I mediated pathway was asessed using interferon I score (IFN1 score). The score represented the median expression of 5 IFN1-regulated genes (IFI44L, IFI44, IFIT3, LY6E, MX1) measured by quantitative real-time PCR. Patients with CNO were treated with standard 3-day regimen (1 mg/kg/day). We measured interferon score before pamidronate (Day 0, n=9) and after (Day 3, n=7. The Local Ethics Committee approved the study at St. Petersburg State Pediatric Medical University (protocol No. 10/8 of 10/23/2017).

Results: median Interferon score was 1.09 (0.96; 1.67) in CNO patients before BF. In 6/7 CNO patients interferon score increased after pamidronate treatment (p=0.015). The median interferon score after pamidronate became 3.06 (0.87; 4.9, p=0.043).

Conclusion(s): It could be supposed that BF may activate interferon-I-regulated pathway and could be a reason of flu-like syndrome. Further investigations are required.

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P006

The blood-cerebrospinal fluid barrier, an important regulator of vitamin D metabolite concentrations in the central nervous system

Sieglinde Zelzer[a], Meinitzer Andreas[a], Herrmann Markus[a], Goessler Walter[b], Enko Dietmar[a]
[a]*Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria*
[b]*Institute of Chemistry, University of Graz, Graz, Austria*

Background/Introduction: Vitamin D, an established regulator of calcium and phosphate metabolism, has important functions in the central nervous system (CNS). The impact of blood-cerebrospinal fluid (CSF) barrier (BCB) integrity on vitamin D metabolite concentrations in CSF was not investigated until now.

Purpose: This study analyzed vitamin D metabolite concentrations in serum and CSF in relation to BCB function.

Methods: Albumin, 25-hydroxyvitamin D (25(OH)D), 24,25-dihydroxyvitamin D_3 ($24,25(OH)_2D_3$), and 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) were measured in 292 pairs of serum and CSF samples. The CSF/serum ratios were calculated for all analytes (Q_{ALB}, $Q_{25(OH)D}$, $Q_{24,25(OH)2D3}$, $Q_{1,25(OH)2D}$) and correlated with serum 25(OH)D concentrations.

Results: Median (IQR) serum concentrations of 25(OH)D, $24,25(OH)_2D_3$ and $1,25(OH)_2D$ were 63.8 (43.4-83.9) nmol/L, 4.2 (2.2-6.2) nmol/L, and 130.3 (92.6-224.3) pmol/L, respectively. The CSF concentrations of all metabolites were 3.7, 3.3, and 8.7 % of the serum concentrations. Low serum 25(OH)D concentrations were associated with higher $Q_{25(OH)D}$ and $Q_{24,25(OH)2D3}$, whereas $Q_{1,25(OH)2D}$ remained constant.

In 117 patients with BCB dysfunction, the CSF concentrations of all vitamin D metabolites were higher than in 175 individuals with intact BCB. Furthermore, these patients showed stronger associations between $Q_{25(OH)D}$ and Q_{ALB} as well as $Q_{24,25(OH)2D3}$ and Q_{ALB} (β-coefficients 0.847 and 0.866; p-values < 0.001). The regression between $Q_{1,25(OH)2D}$ and Q_{ALB} was not related to BCB function (β-coefficient 0.126; p = 0.359).

Conclusion(s): The concentrations of 25(OH)D, 24,25(OH)2D3 and 1,25(OH)2D are lower in CSF than in serum. Low serum 25(OH)D concentrations are associated with an increased passage of 25(OH)D and 24,25(OH)2D3 across the BCB. The adequate supply of the CNS

with active 1,25(OH)2D may be controlled through intracerebral production.

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P008

Distinct ex vivo biomarker profiles of calcified and non-calcified acetabular labrum tissues in primary hip osteoarthritis

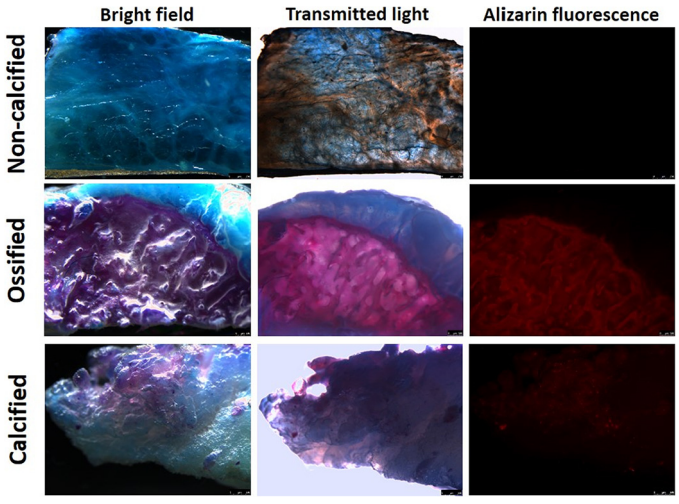
Alexander Antoniadis[a], Julien Wegrzyn[a], Elke Moradpour[b], Thomas Hügle[b], Jeroen Geurts[b]
[a]Lausanne University Hospital, Orthopaedics, Lausanne, Switzerland
[b]Lausanne University Hospital, Rheumatology, Lausanne, Switzerland

Background/Introduction: Acetabular labrum is a ring-form fibrocartilage tissue playing a fundamental role in hip joint stability. Degeneration of the labrum involves chondrocyte apoptosis, macrophage infiltration and calcification and is thought to promote progression of primary hip OA. While several histological studies have documented morpological tissue changes, biochemical changes have not been extensively reported.

Purpose: To characterize biomarker secretion of explanted human labrum tissues under basal and inflammatory conditions.

Methods: Labrum specimens were obtained from patients undergoing total hip arthroplasty (n=11, mean age 65.5, 3 women) and subjected to explant culture for one week in the presence or absence of an inflammatory stimulus (1 μg/mL LPS). Secretion of Aggrecan (ACAN), Cartilage Oligomeric Matrix Protein (COMP), MMP13, Pro-Collagen-Ia and IL-6 was assessed by ELISA. Tissue proteoglycans and calcification were evaluated by whole mount Alcian blue/Alizarin red staining followed by ethyl cinnamate-based optical clearing and fluorescence microscopy.

Results: Whole mount staining for proteoglycans and calcium revealed different degrees of calcification of labrum tissues (***Figure***). Five samples contained numerous calcified nodules and one sample was completely ossified. Non-calcified specimens displayed strong proteoglycan tissue staining interspersed with fibrous tissue. Labrum tissues secreted low levels of IL-6 and ACAN (5-50 ng/gr tissue), moderate levels of MMP13 and COMP (100-300 ng/gr tissue) and high levels of Pro- Collagen-Ia (~900 ng/gr tissue). Subgroup analysis revealed significantly higher Pro-Collagen-Ia (1170+230 vs. 596+305) and MMP13 (157+22 vs. 60+15) secretion by calcified labrum specimens. Inflammatory stimulation led to a ~10-fold upregulation of IL-6 and ~2-fold dowregulation of Pro- Collagen-Ia secretion, in both non-calcified and calcified specimens.



Conclusion(s): Biomarker profiling of degenerative acetabular labrum tissues revealed distinct secretion patterns in non-decalcified and calcified specimens, displaying elevated Pro-Collagen-Ia and MMP13 secretion by the latter. Due to its close proximity to articular cartilage, secreted mediators from degenerated labrum might contribute to or even accelerate the development of hip OA.

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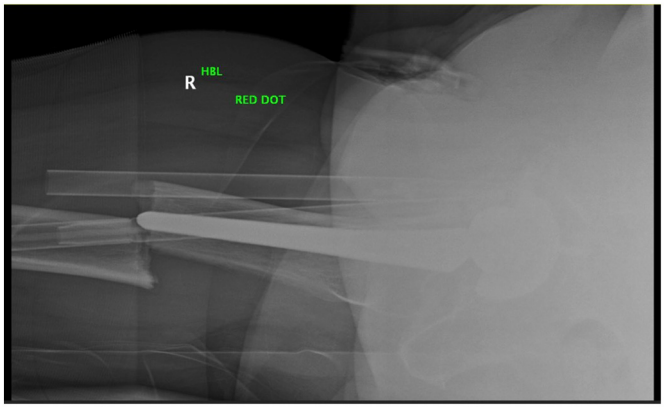
P009

Bisphosphonates (BP) as a potential contributor to prostheses related stress riser fractures

Atef Michael[a], Sohail Querishi[a], Nonyelum Obiechina[b], Imran Chaudary[a]
[a]Russells Hall Hospital, Ortogeriatric Unit, Dudley, United Kingdom
[b]Queen's Hospital, Geriatric Medicine Department, Burton on Trent, United Kingdom

Background/Introduction: Case report: A 68-y-old male had an accidental fall and had right thigh pain. He had right THR for OA two years earlier, left DHS seven years prior to admission. He was compliant on Alendronate for the last seven years.

On admission XR (fig) showed fracture of mid-shaft right femur through the lower aspect of the right hip prosthesis.



Discussion: XR characteristics of the fracture and long duration on Alendronate suggested a BP related AFF. Occurrence of the fracture just through the lower aspect of the hip prosthesis could be due to the impact of the metal prosthesis creating stress riser at this area.

Stress riser is “a mechanical defect in bone, which concentrates stress in an area and increases the risk of failure of the bone at that site”. A stress-riser fracture develops when stress in a prosthesis is higher than that in the surrounding bone. In patients with hip implant there is a stress riser just distal to the implant, predisposing this area to fracture.

BP inhibit osteoclast function, increase bone mineralisation (stiffer bones) however they increase bone hardness (inelastic bones), reduce toughness (reduced ability to absorb energy) and result in accumulation of micro-fractures leading to diminished mechanical strength of bone.

In patients with surgical repair of hip fracture who are on BP we think that a combination of the biomechanical impact of the prosthesis (stress riser) and the effect of BP on the properties of bone, increase the risk of stress riser fractures.

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P011

Bone repair of the rat calvarial defect after application of the xenogeneic biomaterials and dentin

Ana Terezija Jerbić Radetić[a], Željka Perić Kačarević[b], Sanja Zoricic Cvek[a], Igor Erjavec[c], Hrvoje Omrčen[a], Olga Cvijanović Peloza[a]
[a]*Medical Faculty- University of Rijeka, Department of Anatomy, Rijeka, Croatia*
[b]*Faculty of Dental Medicine and Health Osijek, Department of Anatomy Histology- Embryology- Pathology Anatomy and Pathology Histology, Osijek, Croatia*
[c]*Medical Faculty Zagreb, Laboratory for the mineralized tissues, Zagreb, Croatia*

Background/Introduction: Nowdays in oral implantology, natural biomaterials are available, which can be autograft and xenograft. Autologous dentin has been increasingly used in the process of alveolar preservation. Autologous dentin in GBR comes as a natural choice considering that it has the most similar composition of organic and inorganic substances with bone tissue. The presence of BMPs in dentin indicates its pronounced osteoinductive properties. Rapidly absorbable biomaterial can disappear before osteoconduction of osteogenic cells and generate new bone formation, whilst, non-resorbable biomaterials, prevent primary osteogenesis. There is a tendency to use new biomaterials based on magnesium alloy. The reason is that magnesium is biodegradable and it is expected that the biomaterial enriched with magnesium alloy could be completely degraded.

Purpose: The aim was to compare 3D parameters of the bone specimens after application of a new xenogeneic biomaterial (Cerabone with magnesium) with the devitalized dentin, Cerabone and Cerabone with autologous bone.

Methods: After calvarial bone tissue specimen was obtained at days 7, 15 and 30, micro-CT device (Skyscan 1076 micro-CT high resolution in vivo scanner) was used to obtain 3D parameter values: BV/TV (%), Tb. Th (mm), Tb.N (1/mm), Tb.Sp (mm).

Results: The results of the bone volume BV/TV (%) are presented at the figure 1. Overall BV/TV score, no matter which biomaterial was implanted, revealed the highest value at day 15 (53,19 %), which was statistically significant compared to days 7 (27,7 %) and 30 (36,77%), $p<0.01$.

Conclusion(s): The highest values of the newly formed bone were found for dentin and Cerabone with the autologous bone.

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P012

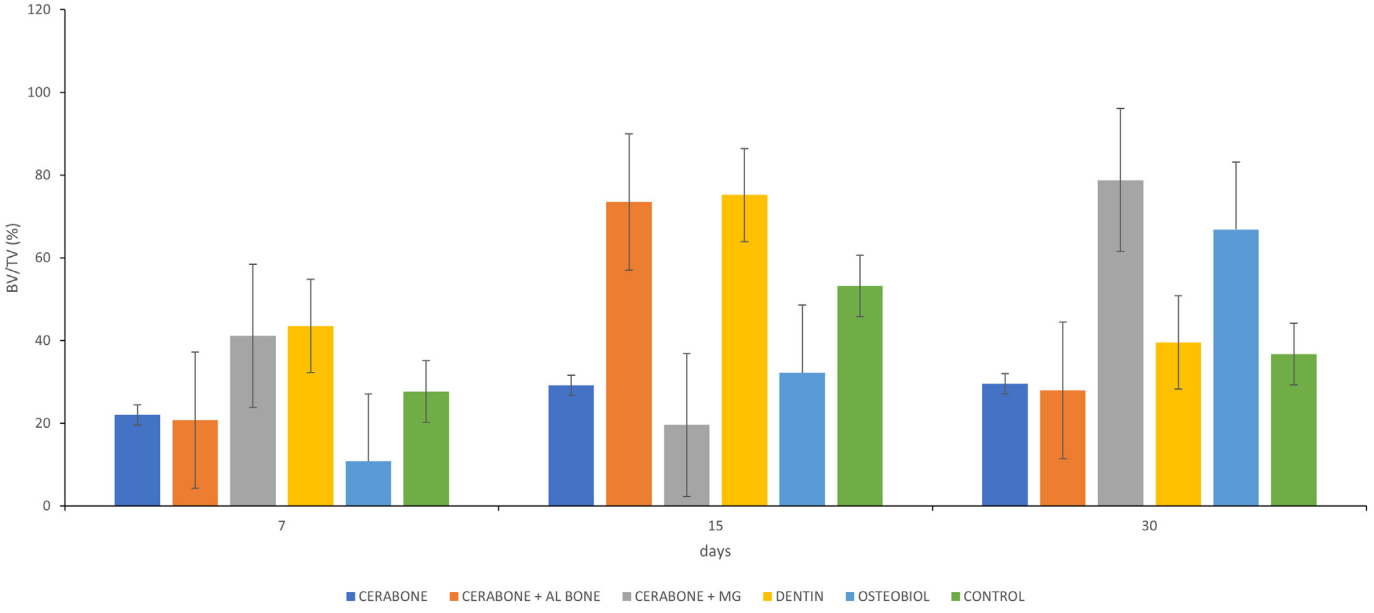
Evaluation of segmentation methods for bone implant screws on in-vivo µCT data in the MgBone study – methodological evaluation of performance and repeatability for screw materials

Timo Damm[a], Jaime Pena[a], Jana Humbert[a], Mirko Gerle[b], Olga Will[a], Reinhard Barkmann[a], Claus-Christian Glueer[a]
[a]*University-Hospital Schleswig-Holstein, Department of Radiology and Neuroradiology- Section Biomedical Imaging- Molecular Imaging North Competence Center MOIN CC, Kiel, Germany*
[b]*University-Hospital Schleswig-Holstein, Department of Oral and Maxillofacial Surgery, Kiel, Germany*

Background/Introduction: 3D segmentation can be carried out either manually or automatically. Manual segmentation suffers from user-dependent bias as well as from varying performance in general. Regarding this aspect automatic and semi-automatic segmentation procedures are of superiority.

Purpose: Considering most metallic screw materials, automatic segmentation is easily carried out using refined, threshold-based algorithms. However, if the density contrast to all surrounding tissues - mineralized bone, bone marrow and muscle and fat – is low, more sophisticated segmentation approaches need to be developed. This is the aim of the project.

Methods: Preclinical *in-vivo* assessment of degradable Mg implants was performed on a Scanco VivaCT 80 at 26µm isotropic resolution. Implants of 2mm diameter and 4mm length were inserted into rat tibia (ethical approv al by local animal experiment board) made from four different materials (degrabable Mg5Gd and Mg10Gd, PEEK, and Ti as control). Besides refined threshold-based segmentation methods, also the performance and repeatability of registration-based approaches using a template screw geometry are investigated on 872 in-vivo µCT screw datasets.



Results: "PAT"-segmentation, based on principal axes transformation works automatically and reliably for high-density objects. For low contrast between screw and surrounding tissue, "REG"-segmentation, based on a two-step, semi-automatic 3D registration is better.

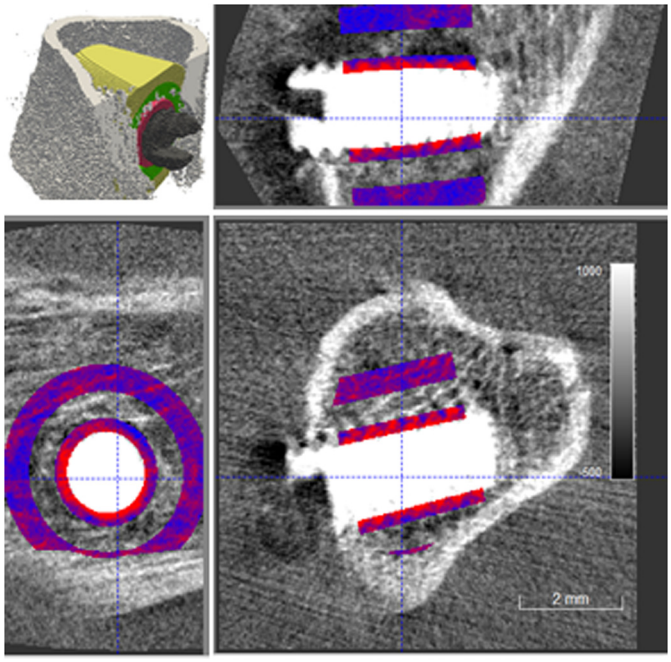


Fig. 1. *Bone implant screw in rat's proximal tibia. 3D segmentations (screw black), orthogonal slices through the screw.*

Material	N in-vivo µCT	Bone-equivalent density of bulk material [mgHA/cm³, mean±SD]	Reg. Method	User interaction / sample
PEEK	219	258±17	REG	~2 min.
Mg5Gd	219	1458±24	PAT	-
Mg10Gd	217	2300±45	PAT	-
Ti	217	2726,76±0 (clipped)	PAT/REG	- / ~2 min.

Conclusion(s): Registration-based segmentation stabilized by a known a-priori implant geometry picks up smaller density – or even textural – contrasts using tailor-made pre-processing and provide better results compared to solely threshold-based approaches. This work was supported by a grant of the Bundesministerium für Forschung und Technologie, 05K16FK3.

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P014

Development of diabetes-sensitive fracture resistance analysis using finite element method to predict diabetic bone fragility and bone quality

Cemre Yavuz[a], Eva Maria Wölfel[a], Katharina Jähn-Rickert[a], Herbert Mushumba[b], Birgit Wulff[b], Klaus Püschel[b], Bjoern Busse[a], Mahan Qwamizadeh[a]

[a]*Universitätsklinikum Hamburg-Eppendorf, Institute for Osteology and Biomechanics, Hamburg, Germany*

[b]*Universitätsklinikum Hamburg-Eppendorf, Institute of Legal Medicine, Hamburg, Germany*

Background/Introduction: Diabetes mellitus is a metabolic disease that is characterized by chronic hyperglycemia. Besides complications such as retinopathy, neuropathy, and cardiovascular diseases, recent studies show that diabetes mellitus also result in a higher risk of bone fracture for both type 1(T1DM) and type 2(T2DM) diabetes patients.

Purpose: The changes in bone structure and composition caused by altered cellular activities may lead to increased bone fragility. Clinical fracture risk assessment tools, such as dual-energy x-ray absorptiometry (DXA) and high resolution peripheral quantitative computed-tomography (HR-pQCT) are based on bone mineral density and microstructure but neglect bone material properties. To determine how diabetes affects bone quality, additional tools such as finite element analysis (FEA) combined with ex vivo experiments are needed.

Methods: For implementation into a high-resolution FE model, IRB approved human cortical bone of the femoral mid-diaphysis subject to T1DM was collected during an autopsy. Beam specimen appropriate for notched fracture mechanics tests with 12x3x2 mm dimension was sawed with a low-speed saw and polished. Micro-CT imaging was performed with 10 µm resolution with a µ-CT 40 (Scanco Medical AG, CH). Micro-CT imaging data sets were transformed into FEM meshes.

Results: Having precise outcomes in terms of porosity occurrence, generated FEM meshes are volume elements instead of voxels, which can be directly mapped from Micro-CT images. With the developed procedure of computational microcrack analysis, fracture properties as the stress intensity factor, fracture toughness as a function of crack growth can be directly calculated from the computational analysis using ABAQUS CAE.

Conclusion(s): The primary FE model serves as a basis to analyze resistance to fracture as a function of crack propagation on prepared and scanned bone structures. To validate computational fracture toughness data and to confirm the model, fracture mechanics tests will be performed. Our approach will contribute to a better understanding of fracture risk in diabetes patients.

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P015

Changes in the microarchitecture of frequently fracturing vertebral bodies in the aging gait

Schroeder Guido[a], Vivell Laura-Marie[b], Andresen Reimer[c], Kullen Claus-Maximilian[d], Schulze Marko[e], Manhart Johannes[f], Martin Heiner[g], Dutzke Mario[a], Hans-Christof Schober[h]

[a]*Warnowklinik, Dept. Orthopedics and Trauma Surgery, Buetzow, Germany*

[b]*University of Rostock, Medical School, Rostock, Germany*

[c]*Westkuestenklinikum Heide- Academic Teaching Hospital of the Univeristies of Kiel-Luebeck and Hamburg, Institute of Diagnostic and Interventional Radiology/Neuroradiology, Heide, Germany*

[d]*Westkuestenklinikum Heide- Academic Teaching Hospital of the Universities of Kiel- Luebeck and Hamburg, Institute of Diagnostic and Interventional Radiology/Neuroradiology, Heide, Germany*

[e]*University of Bielefeld, Institute of Anatomy, Bielefeld, Germany*

[f]*University of Rostock, Insitute for Legal Medicine, Rostock, Germany*

[g]*University of Rostock, Institute for Biomedical Engineering, Rostock, Germany*

[h]*Klinikum Suedstadt Rostock- Academic Teaching Hospital of the University of Rosock, Dept Internal Med IV, Rostock, Germany*

Background/Introduction: Osteoporotic vertebral fractures occur frequently at the lower thoracic and upper lumbar vertebrae.

Purpose: We investigated the structural properties of thoracic and lumbar vertebrae in different age groups.

Methods: 72 core samples (Jamshidi needle 8G) were taken from thoracic vertebrae 8 and 12 (T8, T12) and lumbar vertebrae 1 and 2

(L1, L2) from body donors in two age groups- group A (82±9 years) and group B (36 ± 8 years). The examination was made using a μ-CT (SKYSCAN 1172, RJL Micro &Analytic Company, Germany). We investigated bone volume (BV/TV), trabecular thickness (Th.Th), trabecular separation (Tb.Sp) and the anisotropy (DA). Data was analysed using SPSS, Version 24.0 (SPSS Inc., Chicago, USA). Comparisons between the groups utilized the Kruskal-Wallis test, followed by a pairwise comparison. This study received the approval of the ethics committee of the University of Rostock (**Nr. A 2017-0072**).

Results: Concerning BV/TV, T12 and L2 in group B showed significantly higher results than group A (T12: 14,96 ± 3,92 % vs. 21,32 ± 5,85 %, p = 0.018; L2: 14,66 ± 3,41 % vs. 19,90 ± 4,23 %, p = 0,012), but T 8 and L 1 were not different (p> 0,05).Concerning the Tb.Th. from T12 and L2, the results were significant higher in group B than in group A (T12: 170 ± 17 μm vs. 202 ± 34 μm, p = 0.026; L2: 161 ± 30 μm vs. 196 ± 35 μm, p = 0,037).The DA in group B T8 was significant higher the in group A (T8: 0,28 ± 0,09 vs. 0,37 ± 0,08, p = 0.033). As far as the trabecular separation is concerned there were no significant differences.

Conclusion(s): Decreased bone volume and trabecular thickness in T12 and L2 vertebrae seem to make these vertebrae particularly prone to fracture. Why these particular structural changes occur, however, remains unclear.

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P016

NaQuinate selectively synergises with in vivo mechanical loading stimuli to enhance cortical bone mass and architectural modifications

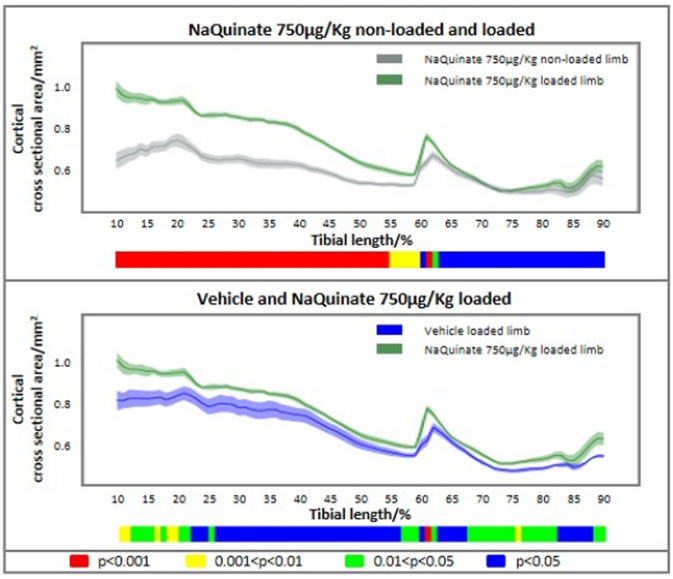
Behzad Javaheri[a], Amy Lock[a], Mark Hopkinson[a], Samuel Monzem[a], Yu-Mei Chang[a], Robin Soper[b], Stephen Hodges[a], Andrew Pitsillides[a]
[a]*Royal Veterinary College, Comparative Biomedical Sciences, London, United Kingdom*
[b]*Haoma Medica Ltd., Drug Development, London, United Kingdom*

Background/Introduction: Generation of bone mass and architecture sufficient to resist fracture, relies on the efficiency of bones' mechanoadaptive response to loading. We have previously demonstrated that NaQuinate maintains bone quantity and quality in a rat ovariectomy model.

Purpose: To evaluate the impact of NaQuinate treatment on bone's response to applied mechanical loading *in vivo*.

Methods: Female 12week-old C57/Bl6 mice (n=8/group) received NaQuinate (750μg/Kg/day) or vehicle 5d/week over 3 weeks. In the final 2 weeks, right tibias were subjected to non-invasive axial loading (12N, 40cycles/day, alternate days); left serving as non-loaded contralateral control. Three days after the last load episode, tibiae were dissected, micro-CT (5μm) scanned and traditional indices of mass and architecture measured in defined trabecular regions and along the entire cortex. Statistical analysis applied linear mixed-effects model followed by Fisher's post-test.

Results: NaQuinate treatment significantly (p<0.05) enhanced cortical responses, generating markedly greater load-related increases in cross-sectional area (CSA)(Fig.1), thickness and J-score across extensive proximal regions. Vitally, clear and significant synergy (p<0.05) was observed between NaQuinate and loading for CSA and J; greater than their distinct additive predicted effect. Comparison of non-loaded tibiae showed significant (p<0.05) NaQuinate-related CSA increases at the midshaft and tibio-fibular junction. NaQuinate treatment also amplified load-related increases in trabecular thickness and generated more vertically-orientated trabeculae than load alone.



Conclusion(s): Synergistic interaction between NaQuinate and loading indicates functional utilisation of bones' mechanostat in the regulation of bone mass and architecture, supporting the notion that NaQuinate can provide a novel therapy in skeletal disorders including osteoporosis.

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P017

Femur specimens from type 1 diabetes mellitus individuals presented with unaffected cortical bone matrix quality, but altered cellular histomorphometry

Sofie Kolibova[a], Eva Maria Wölfel[a], Haniyeh Hemmatian[a], Herbert Mushumba[b], Birgit Wulff[b], Klaus Püschel[b], Björn Busse[a], Katharina Jähn-Rickert[a]
[a]*University Medical Center Hamburg Eppendorf, Department of Osteology and Biomechanics, Hamburg, Germany*
[b]*University Medical Center Hamburg Eppendorf, Department of Forensic Medicine, Hamburg, Germany*

Background/Introduction: Type 1 diabetes mellitus (T1DM) is a chronic metabolic disease of insulin deficiency, presenting with relative risk up to 7-fold higher for hip fractures which cannot be explained solely by lower bone mineral density pointing towards changed bone quality.Due to the scarcity of diabetic bone material, bone quality changes in T1DM have rarely been studied which is essential to elucidate the underlying mechanism of increased fracture risk in T1DM.

Purpose: This study aims to quantify the effect of T1DM on cortical bone quality in terms of microstructure and cellular activity in human femoral specimens using micro-computed tomography (μ-CT) and cellular analyses.

Methods: Femoral cortices of the mid-diaphysis from 25 individuals (T1DM n=9, 52.5±12.0years; control n=16, 51.2±10.2years) were collected during autopsy with IRB approval. Cortical microstructure of the anterior quadrant was determined using μ-CT, while histomorphometry was performed initially at the periosteal region.

Results: Femoral cortical microstructure reflected comparable values for the parameters tissue mineral density, cortical porosity and cortical thickness when using μ-CT (TMD:1076,9±24,2 vs. 1086,59 ±23,35 mgHA/cm³, Ct.Po.: 6,055±4,29% vs. 7,78±6,74%; Ct.Th.: 6175 ±240μm vs. 704±340μm; respectively). However, in only 22% of the T1DM cases osteoblast activity could be identified in the periosteal region,

while 37% of controls showed osteoblast activity. Active osteoclasts were present in 33% of the T1DM cases, while 18% of controls showed osteoclast activity. Moreover, mean values for osteoclast number, osteoclast surface and eroded surface were 3.5-times; 5.7-times; and 6.5-times higher in T1DM compared to controls, respectively.

Conclusion(s): The results of our ongoing study show that only minor changes of the femoral cortical bone architecture occur with T1DM, while a tendency to changed osseous cell activity was noted. Our preliminary data contribute to the understanding of the complex processes contributing to diabetic bone fragility. Additional bone quality factors need to be analysed to elucidate the fracture risk mechanism in T1DM further.

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P019

Hip fracture after hospital discharge from medical wards

Mohanraj Venkatesan, Atef Michael, David Westacott
Russells Hall Hospital, Orthogeriatric Unit, Dudley, United Kingdom

Background/Introduction: In a previous study of 527 hip fracture patients, 4% had the hip fracture while being admitted in hospital and the inpatient mortality in this group was 24% compared to 11 % inpatient mortality of all the hip fracture patients.

Purpose: To study the prognosis of patients who had hip fracture following recent hospital discharge from medical wards.

Methods: A retrospective cohort study of consecutive patients who were admitted with hip fracture in a 4 month period. Patients notes and electronic records were reviewed, and patients discharged from medical ward within 4 weeks prior to sustaining the fracture were identified and studied.

Results: 250 hip fracture patients were admitted in the study period with a mean age of 76 years; 108 were males and 142 females. They had a mean AMT of 7.6.

6% of patients had hip fracture and were admitted within 4 weeks following hospital discharge from medical wards after being medically opimized for discharge. Their mean age was 81 years; 5 male and 10 females. They had a mean of 6.6 in AMT. Out of the 15 patients 53 % fell and sustained fracture within 2 weeks of discharge and 47% within 2- 4 weeks of discharge. The commonest causes for their prior medical ward admission were Confusion 20% or infection; either urinary 13% or cellulitis 13%.

Out of these15 patients 47% died within 2 months post operatively compared to the national 30 day mortality rate of 10% and one year mortality rate 30%.

Conclusion(s): In this study, 6% of hip fracture patients sustain the fracture within one month of hospital discharge from medical wards, This group was older with lower AMT and they had 47% mortality rate within two months.

Elderly patients should have thourough multidiscplinary assessment for the risk of falling and fracture risk before hospital discharge.

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P020

Estrogen receptor α in mature osteoblasts regulate the late stage of fracture healing

Aoi Ikedo, Yuuki Imai
Proteo-Science center- Ehime University, Division of Integrative Pathophysiology, Toon, Japan

Background/Introduction: Estrogen deficiency such as menopause impaired fracture healing. It has been reported that OVX induced estrogen deficiency in mice attenuated fracture healing and the expression ratio of estrogen receptor (ER) α and ERβ changes during the process of fracture healing under estrogen deficiency. Therefore, ERs may be involved in the regulations of fracture healing. However, the roles of ERs in fracture healing process are still unknown.

Purpose: The purpose of this study is the clarification of the significance of ERs during fracture healing using osteoblast-specific ERs knockout mice with drill hole bone regeneration model.

Methods: The osteoblast-specific ERs knockout mice (osteocalcin (OCN)-Cre;ERα$^{f/f}$ and OCN-Cre;ERβ$^{f/f}$) were generated using OCN-Cre mice, and ERα and ERβ flox mice. ERα and ERβ flox mice were used as a control. Fracture surgery was conducted to the tibiae of 8-week-old female mice using drill hole model. The mice were sacrificed at day 10 and 14 after surgery and analyzed by DXA, μCT, and histological analyses. These animal experiments were approved by the Animal Experiment Committee of Ehime University (approval no. 37A1-1/16).

Results: Femoral BMD was significantly decreased in OCN-Cre;ERα$^{f/f}$ mice compared with ERα flox mice ($p = 0.04$) as reported, but there was no difference between OCN-Cre;ERβ$^{f/f}$ and ERβ flox mice. The callus volume at the restricted drill hole site was significantly decreased in OCN-Cre;ERα$^{f/f}$ compared to ERα flox mice only at day 14 ($p = 0.03$) and not at day 10 ($p = 0.45$). In addition to femoral BMD, there is no significant difference in regeneration callus volume between OCN-Cre;ERβ$^{f/f}$ and ERβ flox mice.

Conclusion(s): These results suggested that ERα but not ERβ in osteocalcin-positive osteoblasts might regulate the late stage of fracture healing process.

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P021

Gross morphology of thymus and spleen in rats after formation of defect in the tibia and sodium benzoate intake

Vladyslav Luzin, Irina Belik, Dmitry Astrakhantsev, Nadezhda Mosyagina
State Establishment of Lugansk People's Republic Saint Luka Lugansk State Medical University, Department of Human Anatomy, Lugansk, Ukraine

Background/Introduction: Morphology of the thymus and spleen in case of skeletal injury has not yet been studied.

Purpose: Aim of the study is to test the effects of perforation of the tibia on gross morphology of thymus and spleen in rats after two-month sodium benzoate intake.

Methods: In the study we used 60 adult male rats with the body weight of 200-210 grams. The animals were were distributed into two groups. Group 1 comprised intact animals. Group 2 comprised animals received 1 ml of sodium benzoate by oral gavage at 500 mg/kg for 60 days. After sodium benzoate discontinue, perforation of both tibiae (2.2 mm round opening between metaphysis and diaphysis) in animals was performed. Animals were withdrawn from the experiment on the 3rd, the 10th, the 15th, the 24th, and the 45th day after defect formation. The spleen and the thymus were weighed and measured immediately after removal.

Results: Fracture and sodium benzoate intake resulted in decreased weight of both thymus and spleen. In the period from the 3rd to the 45th day thymus and spleen weight decreased by 19,8%, 18.7%, 17.2%, 14.1%, and 8,8% and by 17,5%, 17,1%, 16,4%, 13,3%, and 7,6% ($p<0,05$ in all cases). Thymus length decrease was observed by the 3rd, the 10th, and the 15th day (by 12.5%, 10.3%, and 9.6%). Spleen length decreased by the 3rd and the 10th day – by 9.3% and 9,1% ($p<0,05$ in all cases). Width and thickness of the thymus and the spleen decreased only by the 3rd day – by 9.8% and 12,9%, and by 8.0% and 10.1% ($p<0,05$ in all cases).

Conclusion(s): Fracture of the tibia after 60-day sodium benzoate intake results in decreased weight of the thymus and the spleen,

which was observed throughout the whole experiment. Dimensions of these organs decrease only by the 3rd day of observation.

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P022

The effects of implantation of hydroxyapatite into the tibia and oral calcium intake on structure of the humeral shaft

Vladyslav Luzin, Artur Koch'yan, Natal'ya Zabolotnaya, Yuliya Venidiktova
State Establishment of Lugansk People's Republic Saint Luka Lugansk State Medical University, Department of Human Anatomy, Lugansk, Ukraine

Background/Introduction: There is a need for medical correction of the skeleton during the healing of bone defects.

Purpose: Aim of the study is to investigate the structure of the humeral shaft after implantation of hydroxyapatite into the tibia and test efficacy of Biomin MK under these conditions.

Methods: 210 male rats with body weight of 135-145 g were thoroughly selected and four groups were formed. Group A comprised intact animals. Group B comprised animals with round openings in both tibiae made for fracture modeling purpose. In the group C the openings were filled with OK-015. In the group C animals with unfilled openings received intragastric Biomin MK at 90 mg/kg per day and the animals of the group D had OK-015 implanted and received the same treatment. Observation terms constituted 7, 15, 30, 60, 90, and 180 days after intervention. HE stained cross-sections of the humeral shafts were measured with the help of light microscopy.

Results: Fracture of the tibia results in increased bone loss and inhibited bone formation in the humeral shaft with manifestations peak registered by the 30^{th} day. After implantation of OK-015, negative effects of fracture lasted to the 15^{th} day yet restoration of the shaft structure was faster. Administration of Biomin MK to the animals with the implants resulted in more expressed changes by the 7^{th} and the 15^{th} days but by the 30^{th} and the 60^{th} day, the external lamellar layer widened by 3.77% and 4.27% as compared to group C, and osteonic layer, internal lamellar layer, and diameter of the osteons enlarged by 3.51%, 6.46%, and 18.64% by the 60^{th} day ($p<0.05$ in all cases).

Conclusion(s): Administration of Biomin MK after implantation of OK-015 into the tibia results in restoration of the humeral shaft structure by the 30th and the 60th day after intervention.

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P023

Angiogenic potential of human mesenchymal stromal cell and peripheral blood mononuclear cell co-cultures

Liina Uusitalo-Kylmälä[a], Ana Carolina Santo Mendes[b], Lauri Polari[c], Katriina Joensuu[d], Terhi Heino[a]
[a]*University Of Turku, Institute of Biomedicine, Turku, Finland*
[b]*University of Coimbra, Faculty of Science and Technology, Coimbra, Portugal*
[c]*Åbo Akademi University, Department of Biosciences, Turku, Finland*
[d]*Tampere University Hospital, Department of Plastic Surgery, Tampere, Finland*

Background/Introduction: Mesenchymal stromal cells (MSCs) are known to stimulate the survival and growth of endothelial cells (ECs), as well as differentiate into pericytes and thereby support blood vessel formation. Since cells with EC-like phenotype are found among the peripheral blood mononuclear cells (MNCs), co-cultures of MSCs and MNCs have been suggested as a potential tool to enhance angiogenesis e.g. in bone regeneration.

Purpose: The aim was to investigate the pro-angiogenic differentiation potential of human MSC-MNC co-cultures in vitro.

Methods: Bone marrow derived human MSCs (2500 cells/cm^2) were co-cultured with MNCs (50 000 cells/cm^2), isolated from peripheral blood of healthy donors. Magnetic-activated cell sorting was used to isolate cells with CD14, CD34 or CD31 surface markers. Co-cultures were analyzed for cell proliferation and morphology, and for various hematopoietic, endothelial and pericyte marker expression by immunocytochemistry, qPCR and flow cytometry. Angiogenic potential was studied with 3D tube formation assay and Incucyte angiogenesis analysis. VEGF secretion was measured with ELISA. Collection of samples followed the Declaration of Helsinki ethical principles.

Results: EC-like, VEGFR1+ and VEGFR2+ spindle-shaped cells were formed in MSC-MNC co-cultures. The cells were $CD14^+CD31^+CD45^+$ and non-proliferative, suggesting to represent myeloid angiogenic cells (MACs). The cells enhanced HUVECs' tube formation and increased the number and length of EC networks and branching points. In co-cultures, cells of MSC- and MNC-origin contained pericyte-like cells with distinct marker profiles. MSC-derived pericytes expressed αSMA, PDGFRβ, NG2, and CD146, while MNCs differentiated into pericyte-like cells expressing CD14, CD146, PDGFRβ, and NG2. VEGF was secreted in MSC-MNC co-cultures and thus mediated, at least partially, the observed angiogenic effect.

Conclusion(s): Differentiation of functional MACs and pericyte-like cells verified the angiogenic capacity of MSC-MNC co-cultures. This indicates that utilization of autologous MSCs and MNCs and their interactions could be of value for improved vascularization e.g., in bone regeneration.

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P024

Chemical composition of ceramics affects the longevity of CRM in posterolateral lumbar fusion achieved by rhBMP6 in autologous blood coagulum with ceramic particles in rabbits

Nikola Štoković[a], Natalia Ivanjko[a], Igor Erjavec[a], Marko Pećin[b], Ana Smajlovic[b], Hrvoje Capak[c], Zoran Vrbanac[c], Jadranka Bubić Špoljar[a], Dražen Vnuk[b], Mihaela Perić[d], Dražen Matičić[b], Hermann Oppermann[e], Slobodan Vukičević[a]
[a]*University Of Zagreb School Of Medicine, Laboratory for Mineralized Tissues, Zagreb, Croatia*
[b]*University of Zagreb Faculty of Veterinary Medicine, Clinics for Surgery-Orthopedics and Ophthalmology, Zagreb, Croatia*
[c]*University of Zagreb Faculty of Veterinary Medicine, Department of Radiology, Zagreb, Croatia*
[d]*University of Zagreb School of Medicine, Center for Translational and Clinical Research, Zagreb, Croatia*
[e]*Genera Research, Genera, Kalinovica, Croatia*

Background/Introduction: Autologous bone graft substitute (ABGS) containing rhBMP6 in autologous blood coagulum (ABC) as a BMP carrier combined with compression resistant matrix (CRM) is a novel therapeutic solution for bone regeneration.

Purpose: The aim of this study was to evaluate synthetic ceramics with different particle size and chemical composition of particles as a CRM in posterolateral lumbar fusion (PLF) rabbit model.

Methods: ABGS implants were prepared as follow: rhBMP6 (125 mg) was added to autologous blood (2,5 mL) which was mixed with synthetic ceramic particles and left to coagulate. Tested ceramic particles differed in particle size (74-420 μm and 500-1700 μm) and chemical composition (TCP and biphasic ceramics containing TCP and HA in 40/60 ratio). ABGS implants (n=6 per group) were implanted bilaterally between lumbar transverse processes (L5-L6) of New Zealand White Rabbits. At the end of the follow up period (27 weeks) animals were killed and spine

specimens were analysed by microCT, histology and biomechanical testing.

Results: Successful spinal fusion was observed in all animals regardless of the size and chemical composition of tested ceramic particles. However, microCT analyses revealed that chemical composition of ceramics determined longevity of CRM. Specifically, TCP ceramics regardless of the particle size were significantly resorbed while particles containing large proportion of HA were only partially resorbed. Osseointegration of newly formed bone with adjacent transverse processes was observed in all specimens. Moreover, biomechanical testing revealed that adjacent transverse processes were rebridged with biomechanically competent bone.

Conclusion(s): A novel ABGS containing rhBMP6 within ABC successfully promoted lumbar fusion with different ceramic particles used as a CRM. However, the chemical composition of ceramics significantly affected CRM resorbability.

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P025

Structural changes of maxilla in adult rats after implantation of hydroxyapatite material OK-015 into the tibia

Vladyslav Luzin, Alexandr Pilavov, Mariya Trufanova, Dar'ya Vasilenko
State Establishment of Lugansk People's Republic Saint Luka Lugansk State Medical University, Department of Human Anatomy, Lugansk, Ukraine

Background/Introduction: Bone damage is accompanied by changes in the dento-jaw system, but studies of the morphology of the maxilla have not been carried out.

Purpose: Aim of the study is to investigate structural changes of the maxilla in adult rats after implantation of hydroxyapatite material OK-015 into the tibia.

Methods: The experiment involved 90 male rats with the body weight of 190-225 g. Animals were separated into three groups as follows: group 1 that control, group 2 that comprised animals with 2.2 mm round opening in the proximal parts of tibiae, and group 3 that comprised animals with the same openings filled with hydroxyapatite material OK-015. On reaching the respective observation term (7, 15, 30, 60, and 90 days after intervention) the animals were withdrawn from the experiment by means of anesthetized decapitation. Maxillae were collected and gross morphometry was performed.

Results: Perforation of the tibia resulted in inhibition of maxilla growth beginning from the 15^{th} day after intervention. Length decreased from the 15^{th} to the 90^{th} day by 2.72%, 3.04%, 3.83%, 4.52%, and 5.52%. Height of the alveolar process decreased by 8.33%, 10.96%, 13.33%, and 12.18%. Also, in the period from the 30^{th} to the 90^{th} day, thickness of the maxilla was smaller than that of the controls by 6.94%, 8.16%, and 9.27%. After implantation, length of the organ by the 7^{th} and the 15^{th} day were smaller than those of the group 2 by 2.10% and 1.39% and thickness – by 4.41% and 7.41%. On the 60^{th} and the 90^{th} days height of the alveolar process exceeded the value of the group 2 by 6.15% and 8.82% ($p<0.05$ in all cases).

Conclusion(s): Implantation of OK-015 into fracture area reduces negative effects of fracture formation of maxilla beginning from the 60th day of the experiment.

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P026

The effects of implantation of ceramic hydroxyapatite into the tibia on gross morphology of the adrenal glands

Vladyslav Luzin, Irina Solov'yova, Sergey Oberemok, Anna Samokish
State Establishment of Lugansk People's Republic Saint Luka Lugansk State Medical University, Department of Human Anatomy, Lugansk, Ukraine

Background/Introduction: Bone damage is accompanied by changes in the endocrinene condition, but studies of the morphology of the adrenal gland have not been carried out.

Purpose: Aim of the study is to investigate changes of gross morphology of the adrenal glands after implantation of ceramic hydroxyapatite into defect in the tibia.

Methods: In the experiment we used 90 rats with body weight of 190-225 g. Animals were separated into three groups. Group 1 - control, group 2 comprised animals with 2 mm through hole in the proximal parts of both tibiae, and group 3 comprised animals with openings in tibiae filled with hydroxyapatite material OK-015. On the 7^{th}, the 15^{th}, the 30^{th}, the 60^{th}, and the 90^{th} day after intervention, the animals were decapitated and the adrenal glands were excised for further gross measurements.

Results: After defect formation relative weight of the adrenal gland from the 7^{th} to the 60^{th} day was greater than that of the controls by13.19%, 14.33%, 17.50%, and 11.03%. Volume values exceeded those of the controls from the 7^{th} to the 60^{th} day by 8.10%, 12.83%, 12.36%, and 6.81%. After OK-015 implantation, relative weight of the adrenal gland increased in comparison with the values of the group 2 on the 7^{th} and the 15^{th} day by 5.54% and 7.29%. In the same period, volume of the gland increased by 6.76% and 7.87%. On the 60^{th} day, relative weight of the gland decreased by the 90^{th} day by 8.63%. Also, volume decreased from the 30^{th} to the 90^{th} day by 6.10%, 7.52%, and 5.11% ($p<0,05$ in all cases).

Conclusion(s): Formation a bone defect results compensatory hypertrophy of the adrenal gland from the 7th up to the 60th day after intervention. Implantation of OK-015 results in manifestations of alterations in the beginning of the experiment with further faster restoration from the 30th day.

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P027

S-012-1709 treatment showed increment in BMD and biochemical parameters in protein deficient condition in growing rats

Krishna Bhan Singh[a], Pallavi Awasthia[b], Kamini Srivatava[a], Amit Kumar[b], Atul Goel[b], Divya Singh[a]
[a]*Central Drug Research Institute, Endocrinology Division, Sector-10-Jankipuram Extension- Sitapur Road, Lucknow 226031, Utter Pradesh India, India*
[b]*Central Drug Research Institute, Division of Medicinal & Process Chemistry, Sector-10- Jankipuram Extension- Sitapur Road, Lucknow 226031, Utter Pradesh India, India*

Background/Introduction: The major public health problem in India is protein energy malnutrition. In the most crucial stage of growth, this affects the child which can lead to permanent impairment in late years. ASSOCHAM–EY Report in 2017 paints a grim picture of India with respect to the prevalence of malnutrition or undernutrition; 39% are stunted, 21% are wasted and 8% are severely acutely malnourished.

Purpose: We studied that treatment will speed up the recovery of musculoskeletal weakness by replenishing nutrients in a proven rodent PEM model.

Methods: All Experimental rodent diets, normal diet (17%) and protein deficient diet (PD) (10%) were prepared as per recommendations for growing animals, according to the National Institute of Nutrition, Govt. of India. Female growing SD rats were given S-012-1709 at 1.0 mg and 10.0 mg kg−1 day−1 dose by orally for 30 days.

Results: Body weights of the protein deficient and rehabilitated groups were lower than control group. Supplementation of S-012-1709 for 4 weeks showed significantly increased BMD of femur compared with the control group. It appears that treatment has significantly better effect in promoting BMD levels of growing rats compared with the protein deficient group. Mineralization with S-012-1709 treatment at 1.0 mg/kg ($P<0.01$) and 10.0 mg/kg showed significant increase in nodule formation compared with PD group. Protein deficiency was associated with a significant decrease in albumin levels in the PD group when compared with the ND group. Albumin levels were significantly enhanced in treatment groups at 1.0 and 10mg/kg with 10mg/kg dose imparting a better effect. Total proteins were also increased in treatment groups. TGA and ALP were significantly increased in 10mg/kg dose of treatment group ($P<0.001$). So protein deficiency shows significant increase in creatinine in the PD group.

Conclusion(s): We, conclude that S-012-1709 treatment showed increment in BMD and biochemical parameters in protein deficient condition in rats.

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P028

Effectiveness of salvage knee rotationplasty on sarcoma around the knee in adolescents and young adults

Hideyuki Kinoshita, Tsukasa Yonemoto, Hiroto Kamoda, Yoko Hagiwara, Toshinori Tsukanishi, Takeshi Ishii
Chiba Cancer Center, Orthopaedic Surgery, Chiba, Japan

Background/Introduction: Sarcoma is a rare cancer that is most common in the adolescent and young adult (AYA) generation. Various surgeries are performed on sarcomas that develop around the knee, including total knee arthroplasty (TKA), lower limb amputations, and knee rotationplasty (KRP). In recent years, along with perioperative chemotherapy, limb-sparing surgery such as TKA has become more common. However, TKAs have various potential complications, including postoperative infection and durability, often leading the requirement of salvage surgery.

Purpose: This study aimed to investigate the effectiveness of KRP as salvage surgery for uncontrolled infection and implant failure of TKA for sarcoma around the knee in AYA.

Methods: This retrospective cohort study included 33 patients who underwent KRP and were grouped based on the treatment received: initial surgery for sarcoma around the knee (n=18) or as salvage surgery (n=15). Musculoskeletal Tumor Society (MSTS) score, range of motion (ROM) and postoperative results were analyzed.

Results: All 15 patients who underwent salvage KRP had TKA as an initial surgery. Although there were five infections in salvage KRP, which originated from the initial TKA, all cases were controllable, no implant failure occurred. MSTS score and ROM were deemed acceptable in both groups.

Conclusion(s): Salvage KRP is an effective option for uncontrolled complications of initial TKA for sarcoma around the knee.

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P029

Bone mineral density after combined treatment of malignant brain tumors in childhood and adolescence

Olga Golounina[a], Maria Pavlova[b], Zhanna Belaya[c], Irina Glinkina[b], Tatiana Morgunova[b], Nadezhda Mazerkina[d], Olga Zheludkova[e], Valentin Fadeev[b]
[a]*I.M. Sechenov First Moscow State Medical University of the Ministry of Health of the Russian Federation Sechenov University, Clinical Medicine, Moscow, Russia C.I.S.*
[b]*I.M. Sechenov First Moscow State Medical University of the Ministry of Health of the Russian Federation Sechenov University, Endocrinology, Moscow, Russia C.I.S.*
[c]*The National Medical Research Center for Endocrinology of the Ministry of Health of the Russian Federation- Moscow, Neuroendocrinology and Osteopathy, Moscow, Russia C.I.S.*
[d]*The National Medical Research Center of Neurosurgery named after N.N. Burdenko of the Ministry of Health of the Russian Federation, Endocrinology, Moscow, Russia C.I.S.*
[e]*Scientific and Practical Center for Specialized Medical Care for Children named after V.F. Voino-Yasenetsky, Pediatric Oncology, Moscow, Russia C.I.S.*

Background/Introduction: Patients with cancer may be at increased risk of bone loss secondary to disease treatment.

Purpose: To study bone mineral density (BMD) in individuals who have undergone combined treatment of malignant brain tumors in childhood and adolescence.

Methods: Retrospective study was conducted with 59 young adults (31 men; 28 women) who have undergone surgical treatment of malignant brain tumour followed by radiation treatment. Group I consisted of 37 patients, who were treated between the ages of 3 and 16 years. Group II included 22 patients who received treatment between the ages of 16 and 38 years.

Results: GH deficiency according to the results of the insulin hypoglycemia test was diagnosed in 48 patients (81%). The majority of those examined (33 patients (56%)) did not achieve the target growth. Only 5 people from I group was treated with recombinant GH. Correlation analysis demonstrates that age of treatment is the main factor affecting final growth ($r=0{,}619$, $p<0{,}001$). 39 cases of hypothyroidism (66%), 22 cases of secondary adrenal insufficiency (37%) and hypogonadism (19 women; 17 men) were detected. According to the DXA, a decrease of BMD ≤-2.0 SD (Z-score) in L1-L4 was found in 35 out of 59 patients (59%). The BMD in the I group was significantly lower than in patients treated at an older age ($p<0.001$). A moderate correlation was discovered between BMD in L1-L4 at the time of examination and the level of estradiol in women ($r=0.596$, $p<0.05$) and testosterone in men ($r=0.472$, $p<0.05$). Direct correlation between BMD and age of diagnosis was revealed ($r=0.781$, $p<0.01$).

Conclusion(s): The results show that patients need to be monitored annually and for life after combined treatment of malignant brain tumors. The high incidence of osteopenic conditions determines the relevance and need for early diagnosis to prevent further bone loss, reduced bone strength and the risk of fractures.

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P035

Characterization of dormancy in osteosarcoma cell lines cultured in 3D

Camille Jubelin[a,b], Denis Cochonneau[a,c], Javier Munoz-Garcia[a,c], Emilie Moranton[a,c], Marie-Françoise Heymann[a,c], Lisa Oliver[c], Dominique Heymann[a,c]
[a]*Institut de Cancérologie de l'Ouest, Inserm UMR1232 - Team 9, Saint-Herblain, France*

[b]Atlantic Bone Screen, Atlantic Bone Screen, Saint-Herblain, France
[c]Inserm, UMR1232 - Team 9, Saint-Herblain, France

Background/Introduction: Dormancy of cancer cells is a potential mechanism for tumour escape. Dormant cancer cells have a reduced proliferation combined with drug resistance, stemness and/or senescent properties.

Purpose: The aim of the study was to develop an *in vitro* culture model for characterizing the impact of the microenvironment on osteosarcoma cell dormancy.

Methods: MNNG/HOS and MG-63 osteosarcoma cell lines stained with Dil dye, were cultured in three-dimensional environment (3D). Dil^+ cells were considered as dormant cells after long-term culture. Proliferative properties, stemness and gene expression were assessed by flow cytometry, RTqPCR and Nanostring™ approaches.

Results: In 3D, osteosarcoma cells were characterized by a reduced proliferation compared to monolayer culture (2D). After 20 days of culture, the percentage of Dil^+ dormant cells was higher in 3D (MG-63: 62.60%; MNNG/HOS: 30.87%) than in 2D (less than 6% of Dil^+ cells). 3D modified the expression of stemness markers. Indeed, SOX-2 (MNNG/HOS: 1.76-fold±0.19) ; MG-63: 2.76-fold±2.01) were upmodulated and NANOG (MNNG/HOS: 0.36-fold±0.21; MG-63: 0.55-fold±0.37) was downmodulated in 3D compared to 2D. Among the Dil^+ cell subpopulation, the expression of the stemness marker CD133 was increased in a time dependent manner (MNNG/HOS: 3.2-fold; MG-63: 3.6-fold). Transcriptomic analyses revealed a significant downregulation of 18 genes between Dil^- and Dil^+ MNNG/HOS cells. Finally, treatment with cytokines and growth factors modulated the proliferation rate of MNNG/HOS cells. IL-6 and IL-34 treatments resulted in a higher DiI fluorescence intensity (2.2-fold and 2.7-fold respectively) compared to untreated cells. TGF-β reduced the DiI fluorescence intensity by 1.6-fold.

Conclusion(s): 3D cultures facilitate the maintenance of dormant cancer cells characterized by less proliferation properties and by differential gene expression compared to proliferative cells. Soluble mediators identified in the microenvironment modulate the dormancy of osteosarcoma cells. *In vitro* 3D cultures are good models to study cell dormancy in osteosarcoma.

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P036

Interactions between oestradiol and zoledronic acid in the bone microenvironment; physiological changes altering metastatic potential of breast cancer cells in pre- and post-menopausal women

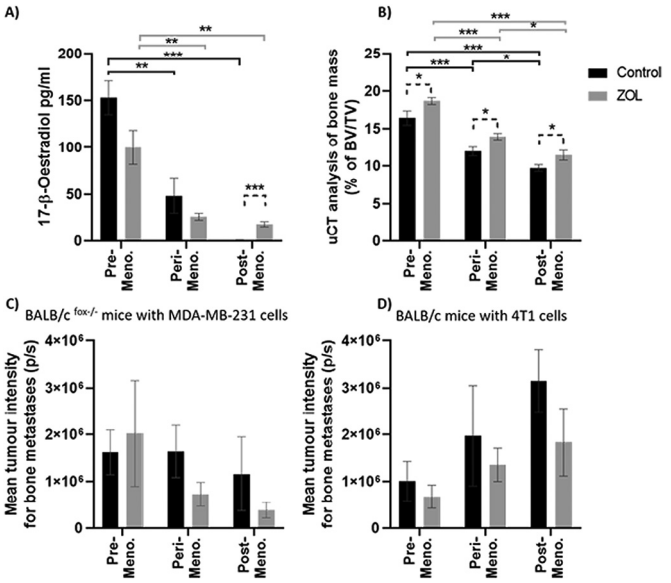
Victor Canuas-Landero[a], Christopher George[a], Diane Lefley[a], Caroline Wilson[b], Penelope Ottewell[a]
[a]The University of Sheffield, Ongology and Metabolism, Sheffield, United Kingdom
[b]The University of Sheffield, Clinical Oncology, Sheffield, United Kingdom

Background/Introduction: Bone is the most common site for breast cancer (BC) metastases. Clinical trials have demonstrated that adding adjuvant zoledronic acid (ZA) to standard of care reduces the risk of BC relapse to bone and other organs in postmenopausal women. Whereas, pre and perimenopausal women experience reduced bone metastasis but increased recurrence outside of bone. Previous research has shown that reduced antitumour effects of ZA seen in premenopausal women are not influenced by inhibin, we therefore hypothesised that these may be driven by interactions between oestrogen and ZA.

Purpose: ZA reduces bone metastasis via inhibition of bone resorption and oestrogen-driven changes to the bone microenvironment drives tumour cells to other sites.

Methods: Mice were ovariectomised (OVX) before receiving 0, 1.38 or 12.5mg/L oestradiol to model post-, peri- and pre-menopausal conditions. All animals received 40ug/day Goserelin to prevent OVX-induced FSH. Animals were randomised to 100ug/kg/week ZA or control. For tumour studies, MDA-MB-231-Luc2 or 4T1-Luc2 cells were injected intra-cardiacly, 4-days after OVX. Bone volume was measured by microCT, bone turnover and oestradiol/FSH by ELISA, histomorphology by osteomeasure and tumour growth by IVIS.

Results: 2-week oestradiol replacement resulted in serum concentrations of 13±10pg/ml, 49±18pg/ml and 153±18pg/ml (post-, peri-, and premenopausal). ZA caused a trend towards reduced serum oestradiol (Fig.1a). Both oestradiol and ZA increased trabecular bone volume ($p=0.001$ and $P=0.05$) (Fig.1b). Oestradiol, alone had no major effects on osteoclasts or osteoblasts. Whereas, ZA reduced osteoclast number ($p=0.05$) and activity ($p=0.001$). ZA administration led to decreased numbers of osteoblasts proportionally to oestradiol concentrations. Oestradiol did not alter the tumour number, size or sites of metastasis. Importantly, in the immune-compromised environment, ZA showed a tendency towards reduction in bone metastasis in the postmenopausal group (Fig.1c,d).



Conclusion(s): Representative menopause model was achieved showing concordance with previous clinical studies. Further analysis of the bone microenvironment in immune-compromised and competent mice is ongoing.

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P037

Studying the role of RANKL in breast cancer and bone metastasis mouse models

Evi Gkikopoulou[a,b], Anthi Kolokotroni[a,b], Vagelis Rinotas[a,b], Melina Dragolia[a], Vasileios Ntafis[a], Martina Rauner[c], Eleni Douni[a,b]
[a]Biomedical Sciences Research Center "Alexander Fleming", Institute for Bioinnovation, Athens, Greece
[b]Agricultural University of Athens, Department of Biotechnology, Athens, Greece
[c]Faculty of Medicine- University Hospital Carl Gustav Carus Dresden, Department of Medicine III, Dresden, Germany

Background/Introduction: Receptor activator of nuclear factor-κB ligand (RANKL) is a multifactorial cytokine that apart from its leading role in osteoclastogenesis and bone resorption, is also involved in mammary epithelial cell proliferation and metastasis.

Purpose: In this study we investigated the effect of RANKL in breast cancer-derived bone metastasis.

Methods: We utilized the E0771 bone metastatic cancer cell line derived from a spontaneous mouse mammary tumour, and assessed its response to RANKL both *in vitro* and *in vivo*.

Results: Our expression analysis in E0771 cells demonstrated the expression of Receptor activator of nuclear factor-κB (RANK) but not RANKL. To investigate whether E0771 cells respond to RANKL, we stimulated these cells with recombinant human RANKL and examined their capacity towards proliferation, Epithelial-to-Mesenchymal Transition (EMT) and migration. Our results revealed that exogenous RANKL treatment did not influence cell proliferation but increased expression of mesenchymal (Vimentin, Twist) and metastatic (MMP9) markers as shown by quantitative PCR. To investigate the effect of RANKL overexpression in the development of mammary tumours and bone metastases we injected 10^5-10^6 E0771 cells through various routes, either systemically or locally to mammary glands and bone, in our TgRANKL transgenic osteoporotic mice overexpressing human RANKL. Since E0771 cells stably express luciferase, we monitored their expansion through *in vivo* bioluminescence imaging, while bone lesions were assessed with X-ray radiation. Preliminary results showed increased bone lesions in TgRANKL mice compared to wild-type controls, when cancer cells were administered directly into the tibia.

Conclusion(s): In conclusion, we showed that the E0771 metastatic cancer cells respond to RANKL, and through the *in vivo* administration of E0771 in TgRANKL mice we are currently investigating the impact of RANKL overexpression by the host microenvironment in tumour progression and bone metastasis.

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P039

Autophagy is a critical process in osteosarcoma cancer stem cells

Marie-Charlotte Trojani[a], Olivier Camuzard[a], Sabine Santucci-Darmanin[a], Sophie Pagnotta[b], Véronique Breuil[a], Georges Carle[a], Valérie Pierrefite-Carle[a]
[a]*Umr e4320 cea/uca, Faculté de Médecine, Nice, France*
[b]*Ccma uca, Faculté de Sciences, Nice, France*

Background/Introduction: Cancer stem cells (CSCs) represent a minor population of cancer cells with stem cell-like properties and appear as a crucial target in oncology as they are at the origin of relapses and resistance to current treatments. Autophagy, which allows the degradation and recycling of cellular components for survival purposes, has been shown to be upregulated in some CSCs, participating in the resistance of these cells. Osteosarcoma (OS) is an aggressive bone cancer which mainly affects children and adolescents and autophagy in OS CSCs has been poorly studied. However, this is a very interesting case because autophagy is often deregulated in this cancer.

Purpose: The aim of our study was to analyze the autophagy level and the consequences of targeting this process in osteosarcoma CSCs.

Methods: First, we used two OS cell lines showing different autophagy capacities to isolate CSC-enriched populations and to analyze the autophagy in basal and nutrient-deprived conditions. Then, we determined the effect of the antipsychotic drug thioridazine which was shown to be able to target the autophagic process.

Results: Our results indicate that autophagy is more efficient in CSCs populations compared to the parental cell lines, suggesting that autophagy is a critical process in OS CSCs. We also showed that the antipsychotic drug thioridazine is able to stimulate, and then impair autophagy in both CSC-enriched populations, leading to autosis, a cell death mediated by the Na+/K+ ATPase pump and triggered by dysregulated accumulation of autophagosomes.

Conclusion(s): Taken together, our results indicate that autophagy is very active in OS CSCs and that targeting this pathway to switch their fate from survival to death could provide a novel strategy to eradicate these cells in osteosarcoma.

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P040

The anti-SLAMF7 elotuzumab enhances ADCC activity with Th1-like γδT cells towards osteoclasts and myeloma cells

Hirofumi Tenshin[a], Takeshi Harada[b], Yusuke Inoue[b], Jumpei Teramachi[c], Masahiro Hiasa[a], Asuka Oda[b], Kotaro Tanimoto[a], So Shimizu[a], Yoshiki Higa[a], Masahiro Oura[b], Kimiko Sogabe[b], Tomoyo Hara[b], Tomoko Maruhashi[b], Itsuro Endo[d], Toshio Matsumoto[e], Eiji Tanaka[a], Masahiro Abe[b]
[a]*Tokushima University, Department of Orthodontics and Dentofacial Orthopedics, Tokushima-Shi, Japan*
[b]*Tokushima University, Department of Hematology- Endocrinology and Metabolism, Tokushima-Shi, Japan*
[c]*Okayama University, Department of Oral Function and Anatomy, Okayama-shi, Japan*
[d]*Tokushima University, Department of Chronomedicine, Tokushima-shi, Japan*
[e]*Tokushima University, Fujii Memorial Institute of Medical Sciences, Tokushima-shi, Japan*

Background/Introduction: Human $V\gamma_9V\delta_2\gamma\delta T$ cells are important effectors in the first-line defense against infection and tumors. Th1-like γδT cells can be expanded *ex vivo* by aminobisphosphonates in combination with IL-2. We reported that such expanded γδT cells effectively target and impair multiple myeloma (MM) cells as well as osteoclasts (OCs).

Purpose: The present study was undertaken to explore the effects of the anti-SLAMF7 therapeutic monoclonal antibody (mAb) elotuzumab (ELO) on cytotoxic activity of the expanded Th1-like γδT cells against OCs and MM cells.

Methods: Human Th1-like γδT cells were expanded from peripheral blood mononuclear cells (PBMCs) by zoledronic acid and IL-2. Human OCs were differentiated from PBMCs with sRANK ligand and M-CSF.

Results: The expanded Th1-like γδT cells highly express CD16, FcγRIIIa, together with perforin and granzymeB, and exerted direct cytotoxic activity towards OCs as well as MM cells. Addition of ELO further enhanced the cytotoxic activity of Th1-like γδT cells against SLAMF7-expressing MM.1S and OPM-2 MM cells but not RPMI 8226 MM cells with marginal SLAMF7 expression, indicating induction of ADCC activity by Th1-like γδT cells in combination with ELO. Importantly, the cytotoxic activity of Th1-like γδ T cells against OCs was also augmented in combination with ELO ($p<0.05$ by ANOVA), although ELO alone did not exert any cytotoxic activity against OCs, indicating ELO's ADCC with Th1-like γδT cells towards OCs. Interestingly, SLAMF7 expression was induced and robustly upregulated in osteoclastic lineage cells during osteoclastic differentiation from monocytes with M-CSF and RANK ligand, and ELO could target preOCs as well as OCs. Furthermore, ELO markedly potentiated the γδT cell-induced cell death against both MM cells and OCs in their cocultures.

Conclusion(s): SLAMF7 is highly expressed in both MM cells and OCs, and OCs as well as MM cells are susceptible to ELO's ADCC with Th1-like γδT cells.

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P043

ZEB1 mediates bone marrow mesenchymal stem cell osteogenic differentiation in vivo and vitro partly through Wnt/β-catenin pathway

Qun Cheng
Huadong Hospital affiliated to Fudan University, Department of Osteoporosis and Bone Disease, Shanghai, China

Background/Introduction: ZEB1 was reported to play an important role in regulating bone development and metabolism. However, the effect of ZEB1 on human bone marrow-derived mesenchymal stem cells (hBMSCs) differentiation, as well as its clinical implication, is unknow.

Purpose: we hypothesized that ZEB1 may be a novel factor for potential therapeutic target for osteogenesis.

Methods: Using quantitative reverse transcriptase-polymerase reaction (qRT-PCR), we examined the expression of ZEB1 during osteogenic differentiation of human BMSCs.Bysmall interfering RNA (siRNA) and lentiviruses in vitro, we demonstrated whether ZEB1 regulates osteogenesis of BMSCs partly via Wnt/β-catenin signaling pathway. We also assayed the effect of ZEB1 knockdown (AAV9-amiR-ZEB1) in OVX mice in vivo. Furthermore, we assessed the ZEB1 expression in bone from normal and postmenopausal osteoporosis patients (PMOP) and its link with bone-related markers and BMD.

Results: The expression of ZEB1 gradually decreased during osteoblastic differentiation of hBMSCs. In vitro, Silencing of ZEB1 stimulated the expression of bone-related genes and increased ALP activity and mineralization of hBMSCs, while overexpression of ZEB1 showed the opposite results. Moreover, siZEB1 activated Wnt/β-catenin pathway. And the increaseof osteogenic differentiation induced by siZEB1 could be partly rescued by inhibitor of Wnt/β-catenin (siβ-catenin). In vivo, knockdown of ZEB1 slowed bone loss of OVX mice to some degree. It was also proven that the expression of ZEB1 negatively associated with bone mass and bone formation genes in patients.

Conclusion(s): In our study, we demonstrated that ZEB1 could regulate the osteogenic differentiation of hBMSCs and silencing of ZEB1 prevents OVX-induced bone loss. therefore, ZEB1 was an essential transcription factor in bone formation and may serve as a potential anabolic strategy for the treatment and prevention of PMOP.

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P044

Osteomodulin impacts positively the bone remodeling process in osteoarthritis

Jérémie Zappia[a], Renée Van der Cruyssen[b,c], Christelle Sanchez[a], Clémentine Lausberg[a], Cécile Lambert[a], Antoine Florin[a], Dirk Elewaut[b,c], Yves Henrotin[a,d]
[a]*University of Liège, musculoSKeletal Innovative research Lab mSKIL-Arthropôle Liège- Center for Interdisciplinary Research on Medicines CIRM, Liège, Belgium*
[b]*Ghent University- Ghent University Hospital, Department of Rheumatology, Ghent, Belgium*
[c]*VIB Center for Inflammation Research, Unit for Molecular Immunology and Inflammation, Technologiepark 927- 9052 Zwijnaarde, Ghent, Belgium*
[d]*Princess Paola Hospital- Vivalia, Department of physiotherapy and functional rehabilitation, Marche- en-Famenne, Belgium*

Background/Introduction: Osteomodulin (OMD) is a protein from the Small Leucine Rich Proteoglycan (SLRP) family. SLRPs are known to perform various functions such as regulating the assembly of the extracellular matrix, growth and cell differentiation. Therefore SLRPs have a crucial role in regulating metabolism and bone development.

Purpose: OMD is highly specific of the bone extracellular matrix. Its production and expression are impaired in subchondral bone sclerosis associated with osteoarthritis. This study aimed to investigate its effects on osteoblasts and osteoclasts.

Methods: Different concentrations of OMD (2.5, 10, 40 and 160 ng/ml), corresponding to the range of OMD concentration found in the supernatant of primary *in vitro* human osteoblast cultures, were tested on human osteoblasts. The mineralization was assessed with the Alizarin red staining at 17-day of differentiation. To investigate the effect OMD on osteoclast differentiation at 4-day of differentiation, OMD (10 and 40ng/ml and 5µg/ml) was mixed to RANKL and M-CSF on primary murine culture. The number of osteoclasts was reported after a TRAP staining. The binding of RANKL-OMD was evaluated with a Solid Phase Binding Assay.

Results: Sclerotic osteoblast cultures showed a strong decrease of mineralization in comparison with non-sclerotic osteoblasts. OMD partially restored the mineralization in the sclerotic osteoblast cultures (Figure 1A). The solid phase binding assay demonstrated the ability of OMD to bind RANKL. The biological consequence of this binding was tested on primary murine osteoclasts in culture. Preliminary results with the pre-mix of RANKL/M-CSF with OMD showed a drastic decrease of the osteoclasts differentiation, and it is even fully blocked at 5µg/ml (Figure 1B).

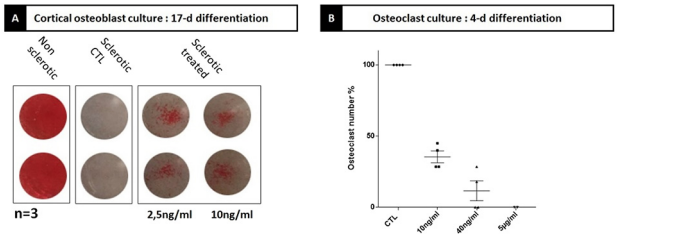

Figure 1: A. Alizarin red staining on cortical osteoblasts after 17 days of differentiation. The cells were treated with OMD during their 3 last days of differentiation. B. Osteoclast culture during 4 days from mouse bone marrow. The cells were treated with M-CSF and RANKL. The OMD was incubated 15 minutes in a premix with the medium supplemented with RANKL and M-CSF. Different concentrations were tested with OMD at 10ng/ml; 40ng/ml and 5µg/ml.

Conclusion(s): OMD is a major proteoglycan in the bone extracellular matrix turnover. It improves the mineralization and inhibits the osteoclast differentiation through the capture of RANKL. Its decrease in osteoarthritic subchondral bone could trigger the sclerosis by initiating or enhancing the imbalance of the bone matrix remodeling.

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P046

Screening of osteogenesis related gene expression in caspase-8 deficient MC3T3-E1 cells

Barbora Vesela[a], Adela Kratochvilova[a], Kamila Rihova[b], Filip Trcka[b], Petr Benes[b], Eva Matalova[a]
[a]*Institute of Animal Physiology and Genetics- CAS, Laboratory of Odontogenesis and Osteogenesis, Brno, Czech Republic*
[b]*Faculty of Science- Masaryk University, Department of Experimental Biology, Brno, Czech Republic*

Background/Introduction: Caspase-8 has been traditionally associated with the extrinsic pathway of programmed cell death. However, recently, knowledge about functions of this cysteine protease keeps expanding beyond apoptosis. This applies also for engagement of caspase-8 in bone development where caspase-8 was abundantly activated in non-apoptotic bone cells.

Purpose: Investigation of caspase-8 in *in vivo* context of osteogenesis is hindered by early embryonic lethality of caspase-8

knock-out and the *in vivo* performed research applies mostly pharmacological inhibitors of the caspase. In this investigation, caspase-8 deficient osteoblastic cells (MC3T3-E1) were achieved by the CRIPSR/Cas9 approach to follow any impact on expression of osteogenesis related genes.

Methods: *Casp8* deficient cells were differentiated using a standard 3-week procedure and after this period, samples were harvested. Osteogenic PCR Array (Qiagen) was used to compare the expression panel in the deficient vs. control cells.

Results: Out of 84 examined genes, significant up-regulation (more than 2-folds) was detected in the case of 3 and down-regulation in the case of 11 genes. The highest increase (30 times) in expression applied for *Gdf10* (Growth/differentiation factor-10), the TGF-beta family member highly related to bone morphogenetic protein-3 which was also impacted by caspase-8 deficiency. The most striking decrease (around 30 times) in expression was found in the case of *Ctsk* (Cathepsin K), essential for normal bone resorption and *Col14a1* (Collagen type 14). Among genes affected by caspase-8 deficiency appeared also *Bglap* (Osteocalcin), one of major osteoblastic markers.

Conclusion(s): The presented results confirm and further specify non-apoptotic functions of caspase-8 in osteogenesis and are a background for additional investigations of the relevant molecular networks.

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P047

FasL induces expression of Mmp13 and Osterix in bone cells

Adéla Kratochvílová[a], Barbora Veselá[a], Eva Švandová[a], Reinhard Gruber[b], Eva Matalová[a]

[a]*Institute of Animal Physiology and Genetics CAS- v. v. i., Laboratory of Odontogenesis and Osteogenesis, Brno, Czech Republic*

[b]*Medical University of Vienna, Department of Oral Biology, Vienna, Austria*

Background/Introduction: Mmps (matrix metalloproteinases) are enzymes with critical roles in extracellular matrix remodelling related to physiological as well as pathological conditions including osteoarthritis, rheumatoid arthritis, and osteoporosis.

Mmp13 is the most intensely studied metalloproteinase regarding cartilage and it is considered as the major catabolic enzyme in osteoarthritis and as a candidate biomarker of the disease and its progression. Mmp13 impacts bone fracture resistance and development of the bone. Another Mmp known to participate in this process is Mmp2. Recently, modulation of Mmp2 expression was demonstrated in FasL deficient bone and in osteoblastic MC3T3-E1 cells.

Purpose: The aim of the presented research was to find out if any similar impact of FasL supplementation can be detected in the case of Mmp13. Since Mmp13 is a direct target of Osterix, the osteoblastic transcription factor, its expression was followed simultaneously.

Methods: The cells were isolated from calva of newborn mice and cultured for 7 days prior to differentiation induction and FasL supplementation to the culture. After 3 days, cells were harvested, RNA isolated, cDNA prepared and the expressions were compared in control and treated cells by qPCR.

Results: The expression of Mmp13 in treated cells rose up to 170 % ($p=0{,}00013$) of control expression and the expression of Osterix to 140 % ($p=0{,}0168$).

Additionally, the expressions of Mmp13 and Osterix were followed after BMP2 and TGFb1 stimulation. While BMP2 caused an increase of Mmp13 (165 %; $p=0{,}00027$) as well as osterix (323 %; $p=0{,}00078$) expression, TGFb1 caused a drop in both, Mmp13 (61 %; $p=0{,}00094$) and osterix (49 %; $p=0{,}00135$).

Conclusion(s): Fas receptor with its ligand, FasL, are traditionally associated with programmed cell death, however, the spectrum of their functions appears much broader, including non-apoptotic pathways in the bone. One of these regulations apparently includes Osterix and Mmp13.

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P048

Analysis of cell cycle related gene expression in Myb deficient mandible – a pilot study

Sabina Stouracova[a,b], Eva Matalova[a,b], Jon Frampton[c], Mary Clarke[c], Premysl Bartos[a], Veronika Oralova[a]

[a]*Institute of Animal Physiology and Genetics- CAS- v.v.i., Laboratory of Odontogenesis and Osteogenesis, Brno, Czech Republic*

[b]*Veterinary University Brno, Department of Physiology, Brno, Czech Republic*

[c]*Institute of Cancer and Genomic Sciences, College of Medical and Dental Sciences, Birmingham, United Kingdom*

Background/Introduction: Myb is a transcription factor well known in regulation of cell cycle and is investigated particularly in haematopoiesis and cancerogenesis.

Purpose: In this research, possible impact on cell cycle regulation in forming mandibular bone was examined using Myb deficient mice. The Myb knock-out is prenatally lethal by the day 15 in the mouse. At this stage, the development of the intramembranous mandibular bone reaches a first complex of vascularized structure containing osteoblasts, osteoclasts and osteocytes.

Methods: The knock-out and wild type samples were fixed in RNA later, then mandibular part of mouse heads were micro-dissected and used for RNA isolation. PCR Array based analysis (Cell Cycle Array, Qiagen) was performed to follow any alterations in expression of cell cycle related genes in the Myb deficient vs. wild type mandibles.

Results: Out of 84 genes within the Array, the affected ones included Ccna1 (CyclinA1), Ccne1 (CyclinE1), Cdkn2a (Cyclin-dependent kinase inhibitor 2A), E2f2 (Transcription factor E2F2), Gpr132 (G protein-coupled receptor 132) and Slfn1 (Schlafen1).

Conclusion(s): There is an increasing evidence about roles of Myb in bone development. In the mandible, delivery of Myb supported bone formation after dental implantations. Our earlier investigations confirmed increasing expression of Myb in the forming mandibular bone. The presented pilot study further indicates possible mechanisms of Myb functions in osteogenesis and is a background for more detailed analyses.

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P049

BMP-2 signaling is enhanced in case of integrin α2β1-dependent collagen upregulation in the musculoskeletal system

Melanie Brand[a], Daniel Kronenberg[a], Jens Everding[b], Melanie Timmen[a], Birgit Mentrup[a], Richard Stange[a]

[a]*Institute for Musculoskeletal Medicine- Westfälische Wilhelms-University, Department of Regenerative Musculoskeletal Medicine, Muenster, Germany*

[b]*Department of Trauma- Hand and Reconstructive Surgery- University Hospital Muenster, Department of Trauma- Hand and Reconstructive Surgery- University Hospital Muenster, Muenster, Germany*

Background/Introduction: The integrins form a family of heterodimeric transmembrane receptors with important function in cell adhesion, signal transduction and interplay of cells with the extracellular matrix. Integrin α2β1 is one out of four collagen-binding integrins and the major receptor for collagen type I in bone tissue. In studies our group showed that integrin α2β1 deficiency favors the expression of collagen type I and supports fracture healing.

Purpose: Recently, we demonstrated first evidence that integrin α2β1-deficient (ITA2$^{(-/-)}$) cells express more BMPs and show an amplified response to the corresponding pathway. Members of the TGF-β superfamily play important roles processing the formation of musculoskeletal tissue and bone. We focus in our study on BMP-2, because with its downstream SMAD1 signaling BMPs are master regulators for bone mineralization and phenotypic stability of osteoblasts leading to higher expression of matrix proteins like collagen type I.

Methods: We analyzed gene expression level of ossification-related genes in isolated wild-type and ITA2$^{(-/-)}$ osteoblasts and chondrocytes by qPCR and phosphorylation level of SMAD1 by immunofluorescence staining during osteoblastic differentiation *in-vitro*. For *in-vivo* analysis we measured the BMP-2 level in mice blood serum by ELISA.

Results: During osteoblastic differentiation, we determined an earlier onset of expression of pro-osteoblastic genes like BMP-2 and BMP-4 in ITA2$^{(-/-)}$ osteoblasts at early stages and in chondrocytes. This correlates to the differentiation states with elevated phosphorylation level of SMAD1 in ITA2$^{(-/-)}$ osteoblasts and chondrocytes. Additionally, we detect a significantly higher BMP-2 concentration in serum of ITA2$^{(-/-)}$ mice after 7 days of fracture healing.

Conclusion(s): This study supports our results pointing on enhanced collagen expression and production in ITA2(-/-) mice which leads to an accelerated fracture repair. We can provide evidence that higher expression levels of BMPs in cells and higher blood serum levels in the corresponding animals are a possible cause of this overall beneficial impact on bone health.

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P051
The evaluation of bone union activity of freeze-dried platelet-rich plasma

Hideyuki Kinoshita[a], Sumihisa Orita[b], Yasuhiro Shiga[b], Kazuhide Inage[b], Seiji Ohtori[b]
[a]*Chiba Cancer Center, Orthopaedic Surgery, Chiba, Japan*
[b]*Chiba University, Orthopaedic Surgery, Chiba, Japan*

Background/Introduction: Platelet-rich plasma (PRP) accelerates bone union in rat model. However, fresh-PRP has a short half-life, making the clinical application of PRP difficult. We suggested freeze-dried PRP (FD-PRP) prepared in advance and investigated its efficacy.

Purpose: This study aimed to evaluate the pharmacological activity of long-term stored FD-PRP *in vitro* and its bone union activity *in vivo*.

Methods: The activation of the downstream protein ERK in osteoblasts, as well as PDGFR by FD-PRP using western blotting was evaluated. Furthermore, osteoblasts proliferation by FD-PRP using MTT assay was analyzed. Spinal posterolateral fusion (PLF) was performed on 8-week-old male Sprague-Dawley rats divided into six groups (n=10 per group): sham, artificial bone, autologous bone, artificial bone + fresh-PRP, artificial bone + FD-PRP preserved 8 weeks, and artificial bone + human recombinant bone morphogenetic protein (BMP) as a positive control. Bone union by radiographs and mechanical strength of spinal segments were evaluated. Furthermore, histological image with hematoxylin and eosin stain of the lumbar spine was analyzed.

Results: The activation of PDGFR and ERK were induced by FD-PRP stimulation. FD-PRP also significantly induced osteoblasts proliferation. Furthermore, PDGFR knockdown attenuated ERK activation by FD-PRP. Comparable radiological bone union was confirmed at 4 weeks after surgery in the FD-PRP groups, which was earlier than in other groups ($p<0.05$). Histologically, the trabecular bone had thinner and more branches in the FD-PRP. Moreover, the biomechanical strength was comparable to that of autologous bone. FD-PRP accelerated bone union at a rate comparable to that of fresh-PRP and BMP by remodeling the bone with thinner, more tangled, and rigid trabecular bone.

Conclusion(s): In conclusion, we showed that long-term stored FD-PRP is pharmacologically active *in vitro* and accelerates bone union in PLF rat models *in vivo*. If FD-PRP is clinically applicable, it could be used to promote bone fusion in bone fracture surgery without intraoperative blood collection.

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P053
Bilirubin increases the viability of osteoclasts and up-regulates osteoclast-related microRNAs

Susana Jurado[a], Albert Parés[b], Pilar Peris[c], Ana Monegal[c], Andreu Combalia[d], Núria Guañabens[c]
[a]*IDIBAPS, Metabolic Bone Diseases and Liver Units, Barcelona, Spain*
[b]*Hospital Clinic i Provincial de Barcelona, Liver Unit, Barcelona, Spain*
[c]*Hospital Clinic i Provincial de Barcelona, Metabolic Bone Disease Unit, Barcelona, Spain*
[d]*Hospital Clinic i Provincial de Barcelona, Orthopedics Unit, Barcelona, Spain*

Background/Introduction: Osteoporosis is a complication of primary biliary cholangitis (PBC), a liver disease resulting in cholestasis and increased circulating bile acids and bilirubin. Bilirubin and litocholic acid (LCA) have harmful effects on osteoblasts and osteocytes *in vitro*. Ursodeoxycholic acid (UDCA), used in the treatment of PBC patients, neutralizes these deleterious actions. The effects of bilirubin and LCA on osteoclasts have not yet been analyzed, although there is evidence of increased bone resorption.

Purpose: The objective was to analyze the effects of bilirubin, LCA and UDCA on the viability of osteoclasts and expression of several microRNAs (miRNAs) that regulate osteoclasts, selected among more than one hundred from the literature.

Methods: RAW 264.7 cells were differentiated to osteoclasts with 30 ng/mL of RANKL. Success in differentiation was assessed by TRAP stain and osteoclasts-specific genes expression analysis. Cells were non-treated (control), treated with the apoptotic agent camptothecin (CAM) and with bilirubin, LCA and UDCA at several concentrations and combinations. Cell viability was measured using WST-1 assay. Expression levels of miR-21a, miR-29b, miR-31, miR-148a, miR-155 and miR-223 were analyzed by real time.

Results: Treatment with bilirubin increased osteoclast viability when compared with the untreated control group (Bi 50 μM of 35% and Bi 100 μM of 80%; $p<0.007$). Cell viability decreased significantly with CAM, LCA and UDCA (500 μM) (59%, 53% and 77%). Combination treatments showed that bilirubin 50 μM with UDCA 10 μM or LCA 10 μM increased viability by 37% and 60%, respectively. Bilirubin increased miR-21 and miR-148a expression compared to the untreated group (115% and 59% respectively; $p<0.007$).

Conclusion(s): Bilirubin increases viability of osteoclasts and this effect counteracts the deleterious actions of LCA and UDCA. Moreover, bilirubin increases expression of miR-21 and miR-148a. Therefore, bilirubin at concentrations found in patients with severe cholestasis

may contribute to increased bone resorption and the development of osteoporosis.

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P056

Impact of Myb deficiency on Rank-Rankl-Opg signalling pathway in osteogenesis

Veronika Oralová[a], Sabina Stouracova[a,b], Jon Frampton[c], Mary Clarke[c], Premysl Bartos[a], Eva Matalova[a,b]
[a]*Institute Of Animal Physiology And Genetics CAS- v.v.i., Laboratory of Odontogenesis and Osteogenesis, Brno, Czech Republic*
[b]*Veterinary University, Department of Physiology, Brno, Czech Republic*
[c]*Institute of Cancer and Genomic Sciences, College of Medical and Dental Sciences, Birmingham, United Kingdom*

Background/Introduction: The central role in bone remodelling belongs to a cytokine triad of TNF family members: the receptor activator of nuclear factor κB ligand (RANKL/TNFSF11), its cognate receptor RANK (TNFRSF11A) and its decoy receptor osteoprotegerin (OPG/TNFRSF11B). Recently, osteogenic impact was reported in the case of Myb, a transcription factor traditionally associated with undifferentiated and cancer cells. Additionally, the first evidence about Myb-Rankl interactions became available.

Purpose: To examine any effect of Myb deficiency on Rankl-Rank-Opg signalling in *in vivo* context, Myb knock-out mice were applied. Such research is hindered by prenatal lethality of the mice (prenatal day 15). However, even at the survival limit, there is available a complex bone structure (containing osteoblasts, osteoclasts and early osteocytes) within the mandible.

Methods: Therefore, molar and incisor parts of the mandibles were isolated from the knock-out mice and their control littermates by micromanipulations, placed into RNA later and used for RNA isolation. The set of samples were investigated by qPCR and expression of Rankl-Rank-Opg compared in the deficient vs. control mandibles.

Results: Statistically significant changes in expression were found in the case of Rankl (3-fold) and Opg (4-fold) while Rank was not affected. The impact on osteoblastic population was confirmed by an additional analysis pointing to modulation of PTHrP (PTH related protein) expression and Bglap (osteocalcin) expression while osteoclastic TRAP was not impacted. Changes in expression of osteocytic Sost were apparent but not significant perhaps due to still early bone development with prevalence of osteoblasts.

Conclusion(s): The results provide the first bone analysis of Myb knock-out mice in *in vivo* context. The findings support the recent evidence about novel functions of Myb in osteogenesis and its impact on Rank-Rankl-Opg signalling.

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P057

Development of biomimetic bone matrices loaded with uranium to study the effect of this actinide on bone cells

Bastien Simoneau[a], Tatiana Gritsaenko[a], Valérie Pierrefite-Carle[a], Gaëlle Creff[b], Agnès Hagège[c], Delphine Farlay[d], Sophie Pagnotta[e], François Orange[e], Xavier Jaurand[f], Christophe Den Auwer[b], Georges Carle[a], Sabine Santucci-Darmanin[a]
[a]*Université Côte d'Azur - CEA, Institut des sciences du vivant Frédéric Joliot - UMR E4320 TIRO-MATOs, Nice, France*
[b]*Université Côte d'Azur - CNRS, Institut de Chimie de Nice - UMR, 7272, Nice, France*
[c]*Université Claude Bernard Lyon 1 - CNRS, Institut des Sciences Analytiques - UMR, 5280, Villeurbanne, France*
[d]*Université Claude Bernard Lyon 1 - INSERM, Lyos - UMR 1033, Lyon, France*
[e]*Université Côte d'Azur, Centre Commun de Microscopie Appliquée, Nice, France*
[f]*Université Claude Bernard Lyon 1, Centre Technologique des Microstructures, Villeurbanne, France*

Background/Introduction: Uranium [U(VI)] is a heavy metal, naturally present in the environment. Due to food, water consumption and anthropogenic activities, general population is chronically exposed to this actinide. The skeleton is the main site of long-term accumulation of this actinide, uranium being trapped into the mineralized bone matrix (Priest et al., 1982). However, interactions of this metal with biological processes involving the matrix and bone cells are still poorly understood.

Purpose: The goal of our work is to get a better understanding of the cellular mechanisms at play in response to uranium storage in bone tissue.

Methods: To mimic bone environment, we developed biomimetic bone matrices containing low doses of natural uranium that we characterized using spectroscopic and microscopic approaches. We used them as a support for the culture and the differentiation of different pre-osteoclastic cell models (RAW 264.7 cells, or C57BL/6 mouse bone marrow precursors) and a substrate for osteoclastic resorption.

Results: Saos-2 osteoblast cells produce a mineralized matrix containing up to 1 μg U(VI) when cultured in the presence of 2 μM U (VI) for 10 days. The ultrastructure of these matrices is not affected by the presence of this actinide, unlike the level of mineralization, which is reduced under these conditions. We observed that U(VI) exerts opposite effects on osteoclastic resorption depending on its concentration and on whether it is in solution, adsorbed or incorporated in the matrix. Furthermore, we have shown that resorption contributes to the remobilization of uranium bound to the bone matrix.

Conclusion(s): These results led us to propose a model for the uranium cycle in bone and its consequences on bone resorption. The relevance of our 3D models to better understand the impact of environmental exposure to uranium on human bone health is also discussed.

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P059

DEC1 deficiency results in accelerated osteopenia through enhanced DKK1 activity and attenuated PI3KCA/Akt/GSK3β signaling

Yang Jian
Nanjing Medical University, School of Basic Medicine, nanjing, China

Background/Introduction: Human differentiated embryonic chondrocyte expressed gene 1 (DEC1) has been implicated in enhancing osteogenesis, a desirable outcome to counteract against deregulated bone formation such as retarded bone development, osteopenia and osteoporosis.

Purpose: The mechanism of DEC1 in the pathogenesis of osteopenia is not well elucidated.

Methods: DEC knockout (KO) and the age-matched wild-type (WT) mice were tested for the impact of DEC1 deficiency on bone development and osteopenia as a function of age.

Results: DEC1 deficiency exhibited retarded bone development at the age of 4 weeks and osteopenic phenotype in both 4- and 24-week old mice. However, the osteopenia was more severe in the 24-week age groups. Mechanistically, DEC1 deficiency downregulated the expression of bone-enhancing genes such as Runx2 and β-catenin accompanied by upregulating DKK1, an inhibitor of the Wnt/β-catenin signaling pathway.

Consistently, DEC1 deficiency favored the attenuation of the integrated PI3KCA/Akt/GSK3β signaling, a pathway targeting β-catenin for degradation. Likewise, the attenuation was greater in the 24-week age group. These changes, however, were reversed by in vivo treatment with lithium chloride, a stabilizer of β-catenin, and confirmed by gain-of-function study with DEC1 transfection into DEC1 KO bone marrow mesenchymal stem cells and loss-of-function study with siDEC1 lentiviral infection into the corresponding WT cells.

Conclusion(s): DEC1 is a positive regulator with *a broad activity* spectrum in both bone development and maintenance, and the osteopenic phenotype accelerated by DEC1 deficiency is achieved by enhanced DKK1 activity and attenuated PI3KCA/Akt/GSK3β signaling.

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P060

Mechanical loading activates YAP/TAZ pathway and chemokines in osteocyte-like cell line MLO-Y4

Martine Cohen-Solal
Inserm U1132 And Universite de Paris, Department of rheumatology, Paris, France

Background/Introduction: Osteocytes are mechanosensitive cells that control bone remodelling in response to mechanical loading. To date, specific signalling pathways modulated by mechanical loading in osteocytes remain elusive. YAP/TAZ, the main effectors of the Hippo pathway, plays a role in mechanotransduction in several cell types and in osteoblastogenesis. We hypothesised that YAP/TAZ signalling mediates osteocyte mechanosensing to target gene of bone remodelling process.

Purpose: We here aimed to investigate the contribution of YAP/TAZ in modulating the gene expression in osteocyte-like cell line MLO-Y4.

Methods: We have developed a three-dimensional (3D) osteocyte compression culture model from MLO-Y4 osteocyte cell line embedded in concentrated collagen hydrogel. Following compression, gene expression was analysed by RNAseq.Validation of gene expression was performed by knocking-down Yaz and Taz though shRNA transfection.

Results: Compared to unloaded conditions, 3D-mechanical loading promoted increased expression of mechanosensitive gene, such as *Ptgs2* (4.7±0.8 vs 38.5±1.8, p=0.001) or *E11/gp38* (22.3±1.7 vs 78.6±12.8, p=0.01)*)*, and a subset of chemokines, including *M-csf*, *Cxcl1*, *Cxcl2*, *Cxcl3*, *Cxcl9* and *Cxcl10* (X3 o 23 fold). Compression induced the translocation of YAP and TAZ into the nucleus and the up-regulation of their target genes and proteins. RNAseq analysis revealed the contribution of several biological processes such as dendrite formation and the modulation of the Cxcl family. Finally, YAP/TAZ knockdown by shRNA partially blunted the increase of *M-csf* (X2 fold) and *Cxcl3* (X8 fold) in response to compression of MLO-Y4 cells, highlighting their role as mediators of mechanically-induced chemokine expression in MLO-Y4 osteocytes.

Conclusion(s): These findings demonstrate that YAP/TAZ signalling is required for osteocyte-like cells mechanotransduction and the control of chemokines expression.

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P061

LIN28a over expression increases Chondrocyte glycolysis to induce chondrocyte reprogramming

Zohra Bouchemla[a], Yohan Jouan[a], Yohan Lionne[a], Lucie Orliaguet[b], fawaz Alzaid[b], Korng Ea[a,c,d], Martine Cohen-Solal[a,d], Eric Haÿ[a,d]
[a]*INSERM, bioscar 1132, Paris, France*
[b]*INSERM, immediab laboratory, Paris, France*
[c]*APHP, hopital Lariboisiere, Paris, France*
[d]*Université de Paris, faculté de medecine, Paris, France*

Background/Introduction: Articular Cartilage have very few regeneration abilities making cartilage damage irreversible. Study of regeneration mechanisms is crucial for osteoarthrosis (OA) therapies. Our previews data showed that Lin28a over expression rebalances the balance between cartilage metabolism and catabolism. the SOX9 reactivation due to the Let7 miRNA inhibition partially explains this reprogramming effect

Purpose: Because Lin28a is also involved in Wolfsburg effect and that chondrocyte mitochondria inhibition activity fetal chondrocyte survey, we analyses the effect of Lin28a over expression chondrocyte energy metabolism and reprogramming

Methods: Primary chondrocyte were transduced with LIN28a or empty vector (Ct), and femoral head harvested from WT or lin28a TG mice, Menisectomy was performed to induce OA.in poth mice

Results: Lin28a in chondrocyte decreased SDHA1 and PDHA expression (respectively +47% and +42%p<0.05 vs Ct) proteins involved in mitochondrial function, and an increased of PFKP (+367% vs Ct p<0.01) a glycolysis associated enzyme. Seahorse analysis, confirmed that LIN28a chondrocyte present more glycolysis activity than Ct (+84%p<0.05). but with the same glycolytic activity and reduced glycolytic reserve (-48% vs Ct p<0.05), indicating that Lin28a chondrocyte use preferentially Glycolysis. Because glycolysis consumes more glucose than oxidative respiration we looked at the ability of chondrocyte to import glucose. We have shown that Chondrocyte highly express Glut1 transporter and Lin28a overexpression dramatically increase its expression in vitro and in vivo. (+105% vs Ct). The absence of glucose as well as Glut1 inhibition totally blunt the pro chondrocyte anabolic effect of Lin28a overexpression (in primary chondrocyte or in femoral head explant). Interestingly we have observed that OA chondrocyte express lower level of Glut1 in mice and human and that Lin28a over expression in mouse sustains Glut1expression even in OA condition.

Conclusion(s): All together these results suggest that glycolysis and glucose intake may play a key role in chondrocyte reprogramming and cartilage regeneration.

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P062

General caspase inhibition in chondrocytes – RNASeq based comparison of OPH and FMK inhibitors

Barbora Veselá[a], Eva Švandová[a], Alice Ramešová[a], Martina Zapletalová[b], Hervé Lesot[b], Jaroslav Doubek[a], Eva Matalová[a]
[a]*University of Veterinary and Pharmaceutical Sciences- Brno- Czech Republic, Department of Physiology, Brno, Czech Republic*
[b]*Institute of Animal Physiology and Genetics- CAS- v.v.i.- Brno- Czech Republic, Laboratory of Odontogenesis and Osteogenesis, Brno, Czech Republic*

Background/Introduction: An RNASeq based approach was used to search for a comprehensive overview of the impact of general caspase inhibition on the transcriptome profile of chondrocytes *in vitro*. These were cultured in micromass, a widely used system for chondrogenesis research.

Purpose: Using the chondrocyte as a model, the impact of general caspase inhibition was investigated in the case of osteogenic as well as autophagic pathways. The aim was to search for the affected genes.

Methods: Cells were obtained from the mouse front limb buds and cultured for 7 days. The impact of two mostly used general caspase inhibitors, Z-VAD-FMK (FMK) and Q-VD-OPH (OPH), was examined and compared.

Results: The experiments revealed a statistically significant impact (more than 2 times) in expression of 542 genes in the FMK samples and

351 genes in the OPH samples compared to controls. The broader effect of the FMK inhibitor corresponds with the expected effect on cathepsins along with caspases. Among the affected genes, 252 were upregulated and 290 were downregulated in the FMK-treated samples, whereas, expression of 163 was increased and expression of 188 genes decreased in the OPH-treated chondrocytes.

The top 10 upregulated genes included Ccl5, Plac8, Cxcl10, Ifi44, Usp18, Isg15, Irf7, Gbp3, Ifit1, Ifih1 in the FMK groups and Ccl5, Plac8, Cxcl9, Ifi44, Usp18, Cxcl9, Gbp3, Ifit1, Isg15, Irf7 in the OPH samples. The top 10 downregulated genes in the FMK treated chondrocytes were Tyrobp, Laptm5, Adgre1, Lcp1, Msr1, Csf1r, Nckap1, Fyb, Trem and Hpgds, in the OPH samples were listed Mrc1, Adgre1, Tyrobp, Trem2, Cyth4, Cd68, Slc37a2, Mmp9, Hpgds, and Ms4a7.

Conclusion(s): Many of the affected genes are associated with pathological conditions, such as osteoarthritis and rheumatoid arthritis, thus emphasizing important functions of caspases in cartilage homeostasis and diseases and pointing to novel regulatory mechanisms.

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P063

Influence of statins on cartilage homeostasis after traumatic impact and during chondrocyte re-differentiation

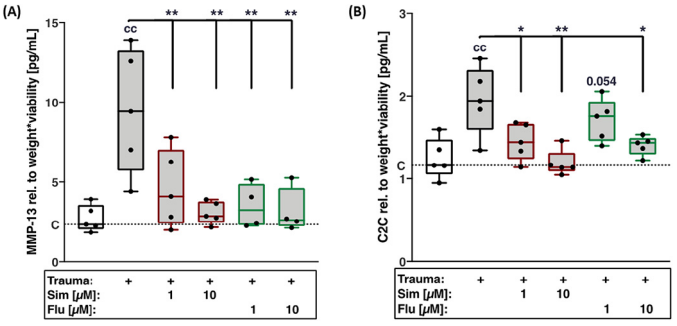
Jana Riegger-Koch, Sai Pulasani, Rolf E. Brenner
University of Ulm, Department of Orthopedics- Division for Biochemistry of Joint and Connective Tissue Disease, Ulm, Germany

Background/Introduction: Due to its anti-inflammatory effects, statins have been discussed as potential therapeutics in osteoarthritic (OA) disease.

Purpose: The present study aims on the potential therapeutic effects of fluvastatin (hydrophile) and simvastatin (lipophile) after cartilage trauma in a human ex vivo model.

Methods: Human cartilage explants were traumatized (0.59 J) using a drop tower system and treated with simvastatin (SIM) or fluvastatin (FLU) (1-10 μM) for 7 d. Cell viability (live/dead assay), secretion of MMP-2 (gelatine zymography) and -13, as well as type II collagen (COL2) biosynthesis and degradation (ELISA) were determined. Influence during re-differentiation of chondrocytes (passages 4) was assessed via Safranin-O and COL2 staining. Statistics: n≥ 5; one-way ANOVA. T= trauma.

Results: Both statins exhibited cell protective effects after T (10 μM: SIM: +17.9%; FLU: +10.6%; each P< 0.001). Moreover, statin therapy significantly suppressed trauma-induced secretion of MMP-13 (Figure 1A) and zymographically detectable amounts of latent and active MMP-2. Subsequent chondroprotection in presence of statins was confirmed by decreased breakdown of COL2 (Figure 1B). However, both statins reduced the biosynthesis of COL2, which was even significant for 10 μM SIM (P= 0.04). Moreover, re-differentiation of chondrocytes was completely impaired by both statins.



Conclusion(s): Despite the suppressive effects during chondrocyte re-differentiation, the tested statins exhibited cell and chondroprotective potential after ex vivo cartilage trauma. Therefore, statin therapy might be beneficial during the acute phase after injury, thus reducing the risk of ongoing cartilage degeneration.

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P065

m6A regulated lncRNA HS3ST3B1-IT1 suppresses the development of osteoarthritis by inhibiting the ubiquitination of HS3ST3B1 protein

Changyan Ma
Nanjing Medical University, School of Basic Medicine, nanjing, China

Background/Introduction: Osteoarthritis (OA) is a common chronic disease with complex etiology and pathogenesis. Long non-coding RNAs (lncRNAs) have been shown to play important roles in the pathogenesis of OA.

Purpose: However, the mechanisms of lncRNAs in the pathogenesis of OA are not fully elucidated.

Methods: In this study, a microarray-based analysis was performed to screen the differentially expressed lncRNAs associated with OA. QRT-PCR was used to detect the expression of lncRNA HS3ST3B1-IT1 in normal chondrocytes and OA chondrocytes. Loss-of-function and gain-of-function assays were used to assess the effects of lncRNA HS3ST3B1-IT1 on extracellular matrix synthesis and degradation as well as cell proliferation and apoptosis. RNA immunoprecipitation, dual luciferase reporter assay, fluorescence in situ hybridization, cellular immunofluorescence, and immunoprecipitation assay were performed to elucidate the underlying molecular mechanisms.

Results: The results showed that HS3ST3B1-IT1 and its target gene HS3ST3B1 were down-regulated in OA cartilage tissues. Both HS3ST3B1-IT1 and HS3ST3B1 in chondrocytes were able to promote ECM synthesis, inhibit ECM degradation, promote chondrocyte proliferation and inhibit the apoptosis.

Conclusion(s): HS3ST3B1-IT1 inhibited the ubiquitination of HS3ST3B1 protein by binding to it, thereby increasing its stability. The m6A demethylase ALKBH5 increased the stability of m6A modified HS3ST3B1-IT1. Data of this study suggest that HS3ST3B1-IT1 and HS3ST3B1 may be novel targets for the prevention and treatment of OA.

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P066

Dmrt2 promotes transition of endochondral ossification by linking Sox9 and Runx2

Kenji Hata[a], Koichiro Ono[b], Eriko Nakamura[a], Sachi Kobayashi[a], Yoshifumi Takahata[a], Tomohiko Murakami[a], Riko Nishimura[a]
[a]*Osaka University Graduate School of Dentistry, Biochemistry, Suita, Japan*
[b]*Nippon Medical School, Department of Orthopedics, Tokyo, Japan*

Background/Introduction: Endochondral bone formation is fundamentally required for skeletal development. During endochondral bone formation, chondrocytes undergo a series of differentiation steps and coordinated transition from proliferating to hypertrophy is critical to advance skeletal development. It has been shown that the cooperation between Sox5, Sox6 and Sox9 is critical in early chondrogenesis and that Runx2 plays primary role in chondrocyte hypertrophy. However, Sox5/6/9 fails to directly modulate Runx2 expression, suggesting the presence of a molecule which links Sox5/6/9 to Runx2.

Purpose: The purpose of this study is to identify the transcription factor involved in the transition from proliferating to hypertrophic chondrocytes,

Methods: We overexpressed Sox5, Sox6 and Sox9 in primary chondrocytes and examined Sox9 target genes by RNAseq analysis. Chondrocyte gene expression was investigated by RT-qPCR. **Results:** We identified the transcription factor *Dmrt2* (double-sex and mab-3 related transcription factor 2) as a Sox9-inducible gene in primary chondrocytes. Epigenetic analysis demonstrated that Sox9 regulates Dmrt2 expression through active-enhancer located 18kb upstream of Dmrt2 gene chromatin status of this enhancer is gradually activated through chondrocyte differentiation.Immunohistochemical analysis revealed that Dmrt2 was strongly expressed in the late proliferating to pre-hypertrophic chondrocytes.Dmrt2 promoted Runx2-dependent expression of *Ihh* and chondrocyte hypertrophy with functional interaction. Of note, the overexpression of Dmrt2 in mouse primary chondrocytes reduced Col2a1 and Aggrecan expression, suggesting that Dmrt2 negatively regulate early stage of endochondral ossification. *Dmrt2*-/- mice exhibited a dwarf phenotype with delayed initiation of chondrocyte hypertrophy.

Conclusion(s): In conclusion, our results suggest that Dmrt2, downstream molecule of Sox5/6/9, was expressed in pre-hypertrophic cartilage and controls endochodral ossification as a linker between Sox5/6/9 and Runx2.Further elucidation of Dmrt2 function in chondrogenesis would contribute to better understanding of the mechanism underlying skeletal development.

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P067

Notch signaling contributes to articular cartilage homeostasis by suppressing differentiation of superficial zone cells

Yasuhide Iwanaga, Fumiko Yano, Taku Saito, Sakae Tanaka
University of Tokyo, orthopedic surgery, Bunkyo-ku, Japan

Background/Introduction: Notch is a single-pass transmembrane cell-surface receptor that plays a crucial role in cell-fate determination and differentiation. We previously reported that Notch-Hes1 pathway promotes osteoarthritis (OA) development; however, there are a few paradoxical reports and the mechanism underlying regulation of joint homeostasis by Notch signaling has not been fully elucidated.

Purpose: To elucidate the expression and functions of Notch signaling in the SFZ.

Methods: First, we isolated superficial zone (SFZ) cells and deep zone (DZ) cells from articular cartilages of 5-day-old mice by sequential collagenase digestion and evaluated Notch related gene expression of SFZ and DZ. For *in vivo* experiment, we generated *Prg4-Cre^{ERT2};Rbpj$^{fl/fl}$* (KO) mice to delete *Rbpj*, an intranuclear cofactor of Notch intercellular domain (NICD), and to suppress Notch signaling activity specifically in the SFZ. After tamoxifen (TM) injection for 5 days at 11 weeks of age, surgical destabilization of medial meniscus (DMM) was conducted in the right knee. We further mated KO mice with *Ai14* reporter mice, and examined fate of *Rbpj*-knockout SFZ cells in non-surgical model, indicated as red fluorescent protein (RFP)-positive cells. For *in vitro* experiment, we cultured femoral head of *Prg4-Cre^{ERT2};Rbpj$^{fl/fl}$* in DMEM , treated by the addition of hydroxytamoxifen, and evaluated markers for chondrocyte maturation. We also generated ATDC5 cells stably express *Hey1* by doxycycline to investigate an inhibitory effect on chondrocyte maturation in ITS differentiation medium.

Results: mRNA levels of Notch-related molecules were highly expressed in SFZ cells, compared to DZ cells. TM-treated KO mice displayed marked OA development at 3 months after DMM surgery. In non-surgical KO mice, SFZ cells were disappeared from SFZ, and broadly distributed in DZ. In vitro experiment revealed that chondrocyte maturation was enhanced by Rbpj knockout, while suppressed by *Hey1* overexpression.

Conclusion(s): Notch signaling is essential for SFZ cells to maintain the undifferentiated nature, and contributes to homeostasis of articular joints.

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P068

Retrospective study of effects of hypomagnesemia on severity of primary hyperparathyroidism

Ding Na[a], Tao Guo[b], Liu ShuYing[a], Wang QinYi[a], Qu XiaoLi[a], Li YongFang[a], OuYang Na[c], Sheng ZhiFeng[a], Yang YanYi[d]
[a]*the Second Xiangya Hospital of Central South University, Department of Metabolism and Endocrinology, Changsha, China*
[b]*the First Affiliated Hospital of Hunan Normal University, Department of surgery, Changsha, China*
[c]*the Second Xiangya Hospital of Central South University, Hospital infection control center, Changsha, China*
[d]*the Second Xiangya Hospital of Central South University, Health Management Center, Changsha, China*

Background/Introduction: The occurrence of hypomagnesemia in patients with primary hyperparathyroidism (PHPT) has been noted previously; however, the association of hypomagnesemia and severity of PHPT remains unknown.

Purpose: To compare the clinical presentation of hypomagnesemia vs non-hypomagnesemia PHPT patients and to evaluate the association of hypomagnesemia with biochemical and clinical manifestations in patients with PHPT.

Methods: This was a retrospective study conducted at a tertiary hospital. We obtained data from 307 patients with PHPT from January 2010 through August 2020. Data on demographics, history, laboratory findings, bone densitometry findings, and clinical presentation and complications were collected.

Results: Among the 307 patients with PHPT included in our study, 77 patients (33/102 [32.4%] males and 44/205 [21.5%] females) had hypomagnesemia. Mean hemoglobin levels in the hypomagnesemia group were significantly lower than those in the normal magnesium group in both males and females. In contrast, patients with hypomagnesemia had a higher mean serum calcium and parathyroid hormone than individuals with normal magnesium. The typical symptoms of PHPT, such as nephrolithiasis, bone pain/fractures, polyuria, or polydipsia, were more common in the hypomagnesemia group. In addition, patients with hypomagnesemia had a higher prevalence of osteoporosis, anemia, and hypercalcemic crisis. Even after adjusting for potential confounders, including age, sex, body mass index, estimated glomerular filtration rate, and parathyroid hormone levels, these associations remained essentially unchanged.

Conclusion(s): Biochemical and clinical evidence indicates that patients with PHPT with hypomagnesemia have more severe hyperparathyroidism than those without hypomagnesemia. Hypomagnesemia is a risk factor for osteoporosis, anemia, and hypercalcemic crisis and may be used as a predictive marker for severity of primary hyperparathyroidism.

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P069

Association between serum magnesium and hemoglobin in primary hyperparathyroidism: A 10-year retrospective study

Ding Na[a], Guo Tao[b], Liu ShuYing[a], Wang QinYi[a], Qu XiaoLi[a], Li YongFang[a], OuYang Na[c], Yang YanYi[d], Sheng ZhiFeng[a]

[a]the Second Xiangya Hospital of Central South University, Department of Metabolism and Endocrinology, Changsha, China
[b]the First Affiliated Hospital of Hunan Normal University, Department of surgery, Changsha, China
[c]the Second Xiangya Hospital of Central South University, Hospital infection control center, Changsha, China
[d]the Second Xiangya Hospital of Central South University, Health Management Center, Changsha, China

Background/Introduction: In the general population, there is a positive association between serum magnesium and hemoglobin levels. No studies have evaluated the association between serum magnesium and hemoglobin levels in patients with primary hyperparathyroidism (PHPT).

Purpose: In this study, we investigated whether there is an association between serum magnesium and hemoglobin levels in PHPT patients.

Methods: Retrospective study of records of demographic and historical findings in PHPT patients who continuously admitted to the Second Xiangya Hospital of Central South University, from January 2010 to August 2020.

Results: Among the 307 hospitalized PHPT patients, 102 were males and 205 were females. A total of 138 patients (49 males [48.0%] and 89 females [43.4%]) had anemia (45.0%). The prevalence of hypomagnesemia was ~25.1%. Average serum magnesium levels were significantly lower in the anemic group than in the non-anemic group in both males and females (0.88 ± 0.19 vs. 0.75 ± 0.17 mmol/L, $P < 0.05$ and 0.89 ± 0.16 vs. 0.80 ± 0.19 mmol/L, $P < 0.05$, respectively). General regression analysis showed that serum magnesium was positively correlated with hemoglobin ($P < 0.001$). Consistently, lower serum magnesium levels were associated with lower hemoglobin levels after adjusting for demographic data, serum calcium, albumin, eGFR, and PTH in PHPT patients ($P = 0.001$).

Conclusion(s): Hypomagnesemia is a common electrolyte disorder in PHPT patients. Hypomagnesemia is associated with lower hemoglobin levels, independent of albumin, serum calcium, eGFR, and PTH in PHPT patients.

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P070
Synchronous post-thyroidectomy hypocalcemia, Claude-Bernard-Horner syndrome and lymphocele after the removal of medullary thyroid cancer on a teenager with multiple endocrine neoplasia type 2A syndrome

Mara Carsote[a], Eugenia Petrova[b], Dumitru Ioachim[c], Anda Dumitrascu[d], Ana Valea[e], Adina Ghemigian[b]
[a]C.I.Parhon National Institute of Endocrinology & C.Davila University of Medicine and Pharmacy- Bucharest- Romania, Endocrinology, Bucharest, Romania
[b]C.I.Parhon National Institute of Endocrinology & C.Davila University of Medicine and Pharmacy, Endocrinology, Bucharest, Romania
[c]C.I.Parhon National Institute of Endocrinology, Pathology, Bucharest, Romania
[d]C.I.Parhon National Institute of Endocrinology, Imaging & Radiology, Bucharest, Romania
[e]I.Hatieganu University of Medicine and Pharmacy & Clinical County Hospital, Endocrinology, Bucharest, Romania

Background/Introduction: MEN (Multiple Endocrine Neoplasia) type2A, familial autosomal dominant disorder associating medullary thyroid cancer(MTC), pheocromocytoma(PHEO), and less frequently primary hyperparathyroidism(PHP), requires early prophylactically removal of thyroid and prompt adrenalectomy when PHEO is confirmed.

Purpose: We introduce a case of post-operative hypocalcemia associated with unusual complications.

Methods: Case report. Patient' and her parents' consent were obtained.

Results: This is a 13-year old female, with positive RET mutation for familial MEN2A(mother operated for MTC and bilateralPHEO), had a serum calcitonin level 5 times above upper normal (at ultrasound: thyroid nodule of 1cm), negative assays for PHEO and PHP. Total thyroidectomy and central neck dissection was performed with confirmation of a micro-MTC and MTC hyperplasia (21 lymph nodes resection without invasion). After surgery she developed symptomatic hypocalcemia (total calcium of 7mg/dL, N:8.5-10.2mg/dL), low normal parathormone (PTH=14pg/mL, N:15-65pg/mL) requiring oral/intravenous calcium supplements and vitamin D. Also, unilateral miosis, ptosis, and nasal congestion confirmed Claude – Bernard - Horner (CBH) syndrome in association with a right anterior cervical mass with rapid progression within 2-3 weeks. Cervical ultrasound initially showed a hypoechoic mass of 6x2X4.45cm with cystic consistence, confirmed by computed tomography as being well shape, probably a hemorrhagic cyst. Due to continuously volume increase, a fine needle aspiration was necessary(cvasi-complete liquid evacuation) confirming lymphocele followed by CBH mild improvement. Within less than 48 hours the tumor relapsed, a micro-surgical procedure was necessary to stop the leaking. While the calcium metabolism and associated symptoms were rapidly controlled, the neurological complication required almost 3months to recover in addition to a local remnant mass of 2 cm (probably a post-operative granuloma).

Conclusion(s): Complications after thyroidectomy involve hypocalcemia especially in large neck dissections. However, additional neurological complications like CBH related to the surgical procedure itself or to lymphocele are very rare. Key words: hypocalcemia, thyroidectomy, Claude Bernard Horner syndrome, lymphocele, medullary thyroid cancer, multiple endocrine neoplasia

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P071
Vitamin D receptor regulates proliferation and differentiation of thyroid carcinoma via the E-cadherin-β-catenin complex

Yali Ling, Zhongjian Xie
The Second Xiangya Hospital of Central South University, Department of Metabolism and Endocrinology, Changsha- Hunan, China

Background/Introduction: Thyroid cancer has the fastest rising incidence among cancers, especially for differentiated thyroid carcinoma (DTC). The prognosis of DTC is largely depending on the degree of cell differentiation and proliferation. However, whether vitamin D receptor (VDR) plays a role in regulating the proliferation and the differentiation of DTC cells is unclear.

Purpose: The present study sought to determine whether VDR regulates DTC cell proliferation and differentiation via E-cadherin and β-catenin.

Methods: Firstly, thyroid papillary carcinoma cell line K1 and thyroid follicular carcinoma cell line WRO were used to construct adenovirus vector VDR-adv and lentivirus vector VDR-shRNA to overexpress and down-regulate the VDR, respectively. The changes of differentiation marker such as TSHR and TPO were detected by qPCR and Western blot. In addition, cell proliferation was measured by BrdU incorporation assay. In addition, the formation of complexes of VDR, E-cadherin and β-catenin was observed by CO-IP. In vivo, K1

cells stably expressing VDR-shRNA and VDR-adv and control K1 cells were injected subcutaneously into SCID mice.The tumor was isolated and weighed, and the expression of proliferation and differentiation of the tumor was detected by IHC.

Results: We found that VDR was upregulated in DTC tissues compared to the adjacent non-cancerous tissue ($P<0.05$). Overexpression of VDR increased the abundance of membrane E-cadherin protein and E-cadherin/β-catenin adhesion complex and differentiation, decreased proliferation in DTC cells in vitro ($P<0.05$), as well as DTC cell derived xenografts in vivo. In contrast, knockdown of VDR had an opposite effect. Knockdown of E-cadherin abolished VDR-induced suppression of proliferation and enhancement of differentiation of the DTC cells. Knockdown of β-catenin partially reversed the effect of the VDR knockdown.

Conclusion(s): Taken together, VDR inhibits DTC cell proliferation and promotes differentiation via regulation of the E-cadherin/β-catenin complex.

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P073

Complex therapy of a patient with type 2 diabetes mellitus (t2dm) and morbide obesity in rehabilitation

Valeriia Vasileva, Larisa Marchenkova
National Medical Research Center of Rehabilitation and Balneology, National Medical Research Center of Rehabilitation and Balneology, Moscow, Russia C.I.S.

Background/Introduction: Treatment of morbid obesity in patients with type 2 diabetes is difficult.

Purpose: The patient T., 43 y.o. addressed with the diagnosis: T2DM (purpose HbA1c <6.5%). Morbid obesity.

Methods: The patient underwent an outpatient rehabilitation program: low-calorie diet, gymnastics in the pool #15, general magnetotherapy # 10, physical therapy in a gym # 15, exercises bike in a gym # 15.

Results: Height 165 cm, body weight (BW) 152 kg, BMI 55.8 kg / m2, waist circumference (WC) 139 cm, hips (HC) 143 cm.HbA1c level of 7.9%, fasting glucose 9.7 mmol /L, total cholesterol (TH) 7.4 mmol / L, triglycerides (TG) 3.08 mmol / L,LDL 4, 8 mmol / L, HDL 1.2 mmol / L, leptin 92.14 ng / ml, 25 (OH) D3 14.6 ng / ml. Bioimpedansometry: fat mass (FM) 81.6 kg, lean mass (LM) 69.4 kg, musculoskeletal mass (SMM) 30.1 kg. Data after completion of the course of treatment: BW 145 kg, BMI 53.3 kg / m2, WC 132 cm, HC 140 cm, BP 128/88 mm Hg, glucose 5.3 mmol / L, TH 6.9 mmol / L, TG 3.03 mmol / L, LDL 4.4 mmol / L, HDL 1.22 mmol / L in serum. Bioimpedansometry: FM 73.4 kg, LM 70.6 kg, SMM 31.8 kg.After 3 months: BW 139 kg, BMI 51.1 kg / m2, WC 128 cm, HC 132 cm, Glucose 5.1 mmol / L, TH 5.9 mmol / L, TG 3.01 mmol / L, LDL 3.52 mmol / L, HDL 1.36 mmol / L, Leptin 36.7 ng / ml, 25 (OH) D3 31.1 ng / ml in serum. Bioimpedansometry: FM 69.3 kg, LM 68.7 kg, SMM 31.0 kg.

Conclusion(s): The clinical case demonstrates the possibilities of complex treatment using non-drug methods and pharmacotherapy for T2DM in combination with morbid obesity.

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P074

Experience of complex rehabilitation of comorbid patient with acute myocardial infarction (AIM) against background of type 2 diabetes mellitus (DM2) and obesity

Valeriia Vasileva, Larisa Marchenkova, Valeriy Sergeev
National Medical Research Center of Rehabilitation and Balneology, Moscow, Russia C.I.S.

Background/Introduction: Rehabilitation of patients after AIM against the background of DM2 with obesity presents difficulties and requires a personalized approach.

Purpose: Presentation of the clinical case is a description of the experience of complex rehabilitation of the patient in the early period of AIM against the background of DM2 in combination with morbid obesity using modern methods of physical therapy and liraglutide therapy.

Methods: *Rehabilitation program:* low-calorie diet, low-intensity laser exposure (over-the-top laser on points) #10, physical exercises in a gym in cardio group # 10, exercises bike in a gym # 10, speleotherapy # 10. Given the transferred AIM, metformin has been cancelled. Liraglutide therapy was initiated at an initial dose of 0.6 mg/day, followed by an increase of 0.6 mg/day. Per week to a therapeutic dose of 1.8 mg/day. There are no undesirable phenomena. Discharged 12 days later to continue his outpatient rehabilitation.

Results: The patient Z., female, 53 y.o.:Coronary heart disease: AIM of the lower wall of the left ventricle with growth of ST. Condition after stenting of the right coronary artery from 21.10.2020.DM2 (HbA1c purpose <7.0%). Obesity. Height 165 cm, body weight (BW) 152 kg, BMI 55.8 kg/m2, waist circumference (WC) 139 cm, hips circumference (HC) 143 cm, blood pressure (BP) 148/98 mm Hg. HbA1c 7.6%, glucose 9.1 mmol/l, total cholesterol (TC) 7.4 mmol/l, triglycerides (TH) 3.08 mmol/l, LDL 4.8 mmol/l, LHL 1.2 mmol/l.

Condition in 30 days: satisfactory, dyshnea decreased, tolerance to physical loads increased. BW 145 kg, BMI 53.3 kg/m2, WC 132 cm, HW 140 cm, BP 124/79 mm Hg. Glucose 5.3 mmol/L, TH 6.9 mmol/L, TG 3.03 mmol/L, LDL 4.4 mmol/L, LDL 1.22 mmol/L in serum.

Conclusion(s): clinical case demonstrates the possibilities of complex rehabilitation of the patient with AIM against the background of DM2 in combination with morbid obesity using physical therapy methods and the use of laraglutide.

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P075

Therapeutic potential of astilbin on diabetes and related secondary complication 'diabetic nephropathy': Therapeutic potential and scientific data analysis of current research work

Dinesh Kumar Patel
Sam Higginbottom University of Agriculture- Technology and Sciences, Faculty of Health Sciences, Prayagraj, India

Background/Introduction: Astilbin is a natural flavonoid compound found to be present in the S. aristolochiifolia, Engelhardtia chrysolepis and Smilax glabra. Astilbin have been well known for their anti-inflammatory activity. Diabetic nephropathy is one of the major complication of all the diabetes patients and responsible for end-stage renal disorders of Human being.

Purpose: Astilbin have been known for their inhibitory potential against carbohydrates-hydrolyzing enzymes which is one of the factors of hyperglycemic condition in the Human being.

Methods: In order to know the effectiveness of astilbin for the treatment of diabetes and related secondary complication, here in the present investigation data analysis of various scientific research works have been performed. However effect of astilbin on α-amylase and yeast α-glucosidase has been also performed through data analysis of scientific work to know their therapeutic potential against diabetes and related complication. All the scientific data have been also correlated with pharmacological activities of astilbin to get better results.

Results: Effects of astilbin against pancreatic α-amylase and yeast α-glucosidase have been investigated to know the importance on diabetes and related complications and revealed significant effect against both enzymes. Molecular docking study also signified their value against α-amylase and α-glucosidase. Another study revealed the inhibitory potential of rat lens and recombinant human aldose reductase which could be used to control and prevent osmotic pressure in the hyperglycemia condition. However some research works suggest that astilbin inhibit connective tissue growth factor (CTGF) which could be potential tools for the treatment of diabetic nephropathy.

Conclusion(s): Data analysis of various scientific researches revealed the importance of astilbin for the treatment of diabetic condition and related secondary complication such as diabetic nephropathy and could be used for the development of better and effective medicine in the future.

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P076

Therapeutic potential of tectoridin in the medicine for their hypoglycemic activity and rat lens aldose reductase inhibitory potential: Medicinal importance through scientific data analysis

Dinesh Kumar Patel
Sam Higginbottom University of Agriculture- Technology and Sciences, Faculty of Health Sciences, Prayagraj, India

Background/Introduction: Tectoridin isolated from the flowers of *Pueraria thunbergiana* and metabolized to tectorigenin by human intestinal bacteria. Tectoridin is a pure phytochemical found to be present in the rhizomes of *Belamcanda chinensis* and flowers of *Pueraria thomsonii*. Aldose reductase is the main enzymes of polyol pathway and inhibition of this enzyme could save peoples having diabetic complications.

Purpose: In the medicine several isoflavonoids have been tested for their aldose reductases inhibitory potential against rat lens to know their biological importance in the diabetes.

Methods: Here in the present investigation biological potential of tectoridin have been evaluated in the medicine through scientific data analysis of current scientific research work for their effectiveness in the treatment of different types of diabetes and related secondary complications. Inhibitory potential of tectoridin against rat lens aldose reductase enzymes have been investigated in the present investigation through scientific data analysis of various research works. Further biological importance of aldose reductase enzyme in the normal and diabetic rats has been also investigated through scientific data analysis.

Results: Scientific data analysis of different research works revealed the medicinal importance of tectoridin in the medicine. From the analysis of current scientific research work it was found that tectoridin are prodrugs which can be converted into tectorigenin through human intestinal bacteria and found to have better hypoglycemic activity. Scientific research signified the biological importance of tectoridin on rat lens aldose reductase which can be used for the treatment of numerous diabetic complications. Some scientific study revealed that orally administered tectoridin in streptozotocin-induced diabetic rats inhibits sorbitol accumulation in the lens and sciatic nerves which signified their importance in the diabetic complications.

Conclusion(s): Scientific database analysis of different research work revealed the therapeutic potential of tectoridin in the medicine and other allied health sectors.

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P077

Anti-hyperglycemic effect of bavachin against diabetes and related complications: Therapeutic role of adiponectin expression and GLUT4 in the medicine

Dinesh Kumar Patel
Sam Higginbottom University of Agriculture- Technology and Sciences, Faculty of Health Sciences, Prayagraj, India

Background/Introduction: Plant and their derived products have been used as raw material for the preparation of different types of medicine to treat human disorders. The fruit of *Psoralea corylifolia* in Korea has been used as traditional medicine for the treatment of numerous health complications including diabetes and related complication.

Purpose: Medicinal importance of bavachin in the medicine and other allied health sectors have been searched and analyzed for the treatment of diabetes and related disorders through scientific data analysis of various scientific works.

Methods: Ethanolic extract of *Psoralea corylifolia* has been proven for their anti-hyperglycemic activity as it showed positive response in the insulin levels, blood glucose and cholesterol levels in the diabetic rats. Detailed pharmacological activities of bavachin in the medicine for their effectiveness against diabetes and associated complications have been investigated through scientific data analysis of different research works. Biological importance of adiponectin expression and GLUT4 translocations in the medicine for the treatment of diabetes and related complications have been also investigated through scientific data analysis of current scientific research works.

Results: Scientific data analysis of the current research work revealed the biological importance of bavachin in the medicine against diabetes and related complications. Data analysis revealed the biological importance of bavachin in the adiponectin expression and secretion in adipocytes. Bavachin also showed positive response on glucose uptake through adipocytes and myoblasts via glucose transporter 4 (GLUT4) translocation.

Conclusion(s): Scientific data analysis of various research works signified the therapeutic benefit of bavachin for the treatment of diabetes and related secondary complication in the medicine.

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P078

Therapeutic benefit and pharmacological importance of asiaticoside for the treatment of human disorders: Role of scientific data analysis in the medicine

Dinesh Kumar Patel
Sam Higginbottom University of Agriculture- Technology and Sciences, Faculty of Health Sciences, Prayagraj, India

Background/Introduction: Plants were used as one of the best source of drugs, food material and general need of human being from very ancient time to till date, as many of the modern drugs have been developed from plants. *Centella asiatica* is well known medicinal herb of India. Asiaticoside is an important pentacyclic triterpenoid saponin of *Centella asiatica* which have anti-inflammatory, anti-oxidant, neuroprotective activity and wound healing properties.

Purpose: To understand the biological importance of phytoconstituents in the medicine for the treatment of various form of inflammatory disorders.

Methods: Medicinal importance and pharmacological activities of asiaticoside have been analyzed through various scientific databases

analysis. Pharmacological activities of asiaticoside have been searched through various scientific databases analysis of literature sources. Numerous research work of literature databases have been analyzed to know the biological importance of various enzymes in the medicine for the treatment of various forms of human disorders.

Results: Literature data analysis revealed the therapeutic benefit of asiaticoside in the medicine for the treatment of various form of human disorders. Literature data analysis of current scientific research work revealed the pharmacological significance of asiaticoside in the medicine and other allied health sectors. Literature data analysis signified the biological importance of asiaticoside against nitric oxide production and showed protective mechanism against oxidative damage and inflammatory disorders. Molecular data analysis of various literature databases revealed the biological importance of various form of enzymes in the biological systems.

Conclusion(s): Literature data analysis signified the biological importance and therapeutic benefit of asiaticoside for the treatment of numerous health complication of human being.

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P080

Comparative metabolic syndrome effect on total and undercarboxylated osteocalcin in non-diabetic women

Marina Bonanno[a], Graciela Brito[b], Dana Watson[c], Liliana Zago[d], Carlos González Infantino[e], Susana N. Zeni[f]

[a]*Institute of Immunology- Genetic and Metabolism INIGEM- National Council for Scientific and Technologic Research CONICET- School of Pharmacy and Biochemistry- Buenos Aires University UBA Clinical Hospital, Metabolic Bone Diseases Laboratory, Buenos Aires, Argentina*

[b]*La Matanza University UNLaM, Nutrition Department, Buenos Aires, Argentina*

[c]*La Matanza University UNLaM, Nutrition Departmen, Buenos Aires, Argentina*

[d]*Buenos Aires University- School of Pharmacy and Biochemistry, Nutrition Department, Buenos Aires, Argentina*

[e]*Buenos Aires University- School of Medicine, Nutrition Department, Buenos Aires, Argentina*

[f]*Institute of Immunology- Genetic and Metabolism INIGEM- National Council for Scientific and Technologic Research CONICET- School of Pharmacy and Biochemistry- Buenos Aires University UBA Clinical Hospital, Metabolic Bone Diseases Laboratory, CABA, Argentina*

Background/Introduction: Bone has a specific function in controlling whole-body glucose homeostasis and insulin resistance through the bone-derived proteins, particularly osteocalcin (ucOCN). Previously, we found that body mass index (BMI) through leptin is related to OCN levels in both sexes.

Purpose: We compare ucOCN and total OCN (tOCN) levels in non-diabetic women having normal glucose and HbA1c levels with different degree of obesity: overweight (OW) or type I, II, III obesity (OB) presented or not MetS.

Methods: Glucose and HbA1c were measured by standard methods; ucOCN (ng/mL), tOCN (ng/mL), leptin (ng/mL), insulin (μUI/L) and CTX (ng/L) by ELISA, and 25hydroxyvitaminD (25OHD) (ng/mL) by a competitive protein-binding method. MetS was established according to WC ≥80 cm and two of the following factors: TG (≥150mg/dL), HDL (<50mg/dL), hypertension (blood pressure≥130/85mmHg) or homeostasis model assessment-insulin resistance index (HOMA-IR>3). HOMA-IR was calculated as: fasting insulin levels (μUI/mL) x fasting glucose (mg/dL)/405.

Results: In almost all degree of obesity, tOCN and CTX levels decreased while ucOCN increased in women having MetS ($p<0.05$). Leptin and 25OHD tended to decrease in women having MetS. In both groups, WC and BMI correlated with ucOCN, while tOCN correlated with CTX levels ($p<0.05$).

	Without MetS			
	OW	OBTI	OBTII	OBTIII
tOCN	32.0 ± 14.5^{b}	22.3 ± 13.1^{ab}	36.5 ± 3.0^{b}	10.7 ± 4.5^{a}
ucOCN	1.0 (0.6-3.4)[a]	2.4 (0.1-4.0)[ab]	2.1 (1.3-4.8)[ab]	4.4 (4.3-4.6)[b]
CTX	517 (195-554)	513 (245-557)	560 (550-577)	472 (137-542)
25OHD	22.9 ± 8.1	21.4 ± 7.9	20.0 ± 7.3	18.2 ± 6.5
Leptina	10.3 ± 5.5^{a}	17.9 ± 11.9^{ab}	23.9 ± 6.5^{abc}	38.8 ± 17.6^{c}
	MetS			
	OW	OBTI	OBTII	OBTIII
tOCN	28.5 ± 12.5^{ab}	24.3 ± 12.1^{ab}	21.2 ± 14.9^{ab}	27.3 ± 12.9^{ab}
ucOCN	0.9 (0.6-1.4)[a]	3.4 (1.1-5.9)[ab]	4.4 (1.9-4.8)[ab]	3.5 (1.2-4.5)[ab]
CTX	279 (205-538)	534 (219-552)	325 (195-559)	546 (268-563)
25OHD	22.4 ± 8.0	22.2 ± 10.1	16.4 ± 2.9	17.5 ± 6.0
Leptina	9.9 ± 6.3^{a}	12.8 ± 2.1^{a}	22.2 ± 10.7^{ab}	27.5 ± 6.9^{bc}

Conclusion(s): Our results suggest that ucOCN levels appear to be associated with several components of MetS and with BMI while bone remodeling was negatively associated with MetS. Supported by CONICET/UBA and PROINCE E006 grant of UNLaM.

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P081

Anti-diabetic activity of flavonoid rich fraction of Pedalium murex in the medicine: Biological importance of aldose reductase in polyol pathway for prevention of microvascular complications

Dinesh Kumar Patel

Sam Higginbottom University of Agriculture- Technology and Sciences, Faculty of Health Sciences, Prayagraj, India

Background/Introduction: *Pedalium murex* is important medicinal plants which have been used for the treatment of various human health complications such as gonorrhea, asthma, headache, diarrhea dysuria, pain and fever in the traditional system of medicine.

Purpose: Biological importances of flavonoidal rich fraction of *Pedalium murex* in the medicine to treat diabetes disorders have been investigated in the present investigation.

Methods: In the present investigation, anti-diabetic activity of flavonoid rich fraction of *Pedalium murex* have been evaluated through various *In-vitro* and *In-vivo* techniques in order to determine the biological potential of *Pedalium murex* in the diabetes and related secondary complications. Phytochemical tests have been performed in the present investigation to know the presence of various active secondary metabolites in the *Pedalium murex*. Healthy Charles foster rats (150-200 g) were taken in the present investigation for determination of anti-diabetic potential and aldose reductase inhibitory potential using rat lens AR enzyme.

Results: Phytochemical analysis revealed the presence of various phytoconstituents in the flavonoid rich fraction of *Pedalium murex* which signified the biological potential of these phytoconstituents in the medicine. HPTLC fingerprinting analysis were performed and revealed the presence of different active phytoconstituents in the flavonoid rich fraction of *Pedalium murex* in the chloroform: methanol (8:2) solvent system. *In-vivo* study data signified the biological potential of flavonoid rich fraction in the medicine for the treatment of diabetes and related secondary complication as it showed better effectiveness in the streptozotocin induced diabetes animal models. Aldose reductase inhibitory potential of flavonoid rich fraction were studied in the rat lens aldose reductase (AR) enzyme and showed significant potential in the

tested model as it showed non-compititive inhibition through the values of Vmax, Km and Ki.

Conclusion(s): Phytochemical analysis confirmed the presence of numerous secondary metabolites which could be useful for the treatment of diabetic and related secondary complication in the medicine.

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P087

A novel MEN1 pathogenic variant in a patient with MEN1 syndrome: Case report

Hani Rjoob[a], Tayyab Khan[b]
[a]*London Health Science Center, Medicine / Division of Endocrinology, London, Canada*
[b]*London Health Science and Center, Medicine / Division of Endocrinology, London, Canada*

Background/Introduction: The multiple endocrine Neoplasia type 1 (MEN-1) is an autosomal dominant syndrome characterized by predisposition for tumors of parathyroid glands, pancreatic islet cells and pituitary gland. MEN-1 gene; the gene causing MEN-1 syndrome, is located on long arm of chromosome 11 (11q13) and it encodes for the protein menin.

Purpose: We report a case of a novel gene mutation for MEN-1 syndrome.

Methods: A 60 year old lady who was diagnosed with primary hyperparathyroidism. She had left inferior parathyroidectomy with pathology reported hypercellular parathyroid tissue. Follow up biochemical testing and parathyroid scan confirmed persistent hypercalcemia with hyperparathyroidism. She had abdominal pain, for which work up was done and showed three pancreatic masses. Fine needle aspiration of large pancreatic body mass confirmed a neuroendocrine tumor. Work up confirmed also presence of sellar mass, suggestive of a non-functioning pituitary adenoma.

Results: Giving this clinical presentation, she had genetic testing, which revealed a heterozygous mutation, MEN1:c.1192delC, which resulted in a frameshift mutation leading to the termination of MEN-1 gene, and could be associated with nonsense mediated decay of the MEN-1 mRNA. To our knowledge, this is the first report of this mutation associated with MEN-1 syndrome.

Conclusion(s): We report a novel heterozygous pathogenic variant of MEN-1 gene, located in exon 9. While more than 1,200 germline mutations in the MEN-1 gene have been identified, to our knowledge, this is the first report linking MEN-1 syndrome with this gene mutation. There could be variability in clinical presentations of MEN-1 syndrome depending on genetic variations, and further monitoring of this patient and her family could help in better characterization of the disease condition due to this mutation.

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P088

WNT16 variants influence site-specific bone mass determination and fracture risk in Maltese postmenopausal women

Melissa M. Formosa, Sarah Ann Mallia, Warren Portelli, Angela Xuereb-Anastasi
University of Malta, Applied Biomedical Science, Msida, Malta

Background/Introduction: Epidemiological studies and translational models have highlighted the importance of WNT16 as a key regulator of bone mineral density (BMD).

Purpose: The study aimed to investigate the effect of two *WNT16* variants, a frameshift rs55710688 (insCCCA) in the Kozak sequence, and a single nucleotide variant rs3801387 (A>G) located in the last intron of *WNT16*, with BMD and fracture risk in the Maltese postmenopausal women.

Methods: Genotyping was performed in 1,045 women from the Malta Osteoporotic Fracture Study using Competitive Allele Specific PCR (rs55710688) and TaqMan® fluorogenic 5' nuclease allelic discrimination (rs3801387). Genotype-phenotype associations were analysed using the Mann-Whitney statistic whereas odds ratios (OR) with 95% confidence intervals [CI] were computed by logistic regression analysis adjusted for confounders.

Results: Genotyping of the *WNT16* rs55710688 and rs3801387 was successful in 1,038 (CCCA=24%) and 1,027 (G=27%) samples respectively. Women with the homozygous reference genotype for both *WNT16* variants had a lower lumbar spine (LS) T-score relative to women with the homozygous alternative genotype (rs55710688 $p=0.035$; rs3801387 $p=0.031$). Risk ratios revealed that homozygosity for the reference alleles was associated with osteoporosis at the LS (rs55710688 adjusted-OR: 2.44 [1.16-5.13]; rs3801387: 2.40 [1.18-4.89]), and all-type of low-trauma fracture risk which was not attenuated by BMD (rs55710688: 2.17 [1.11-4.27]; rs3801387: 1.90 [1.05-3.55]). *WNT16* rs55710688 reference genotype also exhibited a deleterious effect on femoral neck BMD (3.10 [1.05-9.15]). Finally, the haplotype with the reference alleles for *WNT16* rs55710688 and rs3801387 was associated with LS BMD ($p=0.007$) and fracture risk ($p=0.021$).

Conclusion(s): Results indicate that the *WNT16* rs55710688 and rs3801387 variants are possible genetic determinants of site-specific BMD and fracture risk in Malta, which is in line with other epidemiological studies. Our findings support the results of *in vitro* assays and *in silico* modelling demonstrating reduced translational efficiency in the presence of the reference alleles culminating in lower bone formation.

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P089

Functional characterization of CYP1A1 variants identified in patients who sustained bisphosphonate-related atypical femoral fractures

Nerea Ugartondo[a], Monica Esteve[a], Natalia Garcia-Giralt[b], Nuria Martinez-Gil[a], Raquel Rabionet[a], Daniel Grinberg[a], Susanna Balcells[a]
[a]*University of Barcelona, Genetics- Microbiology and Statistics, Barcelona, Spain*
[b]*Hospital del Mar Medical Research Institute IMIM, Musculoeskeletal Research Group, Barcelona, Spain*

Background/Introduction: Atypical Femoral Fractures (AFF) are a possible adverse effect of long-term osteoporosis treatment with nitrogen containing bisphosphonates (BP). Due to the small incidence of AFF, an underlying genetic cause is suspected to increase AFF susceptibility, which might be triggered by BP exposure. Previous studies uncovered *CYP1A1* to be mutated in osteoporosis patients with BP-related AFF. CYP1A1 is involved in drug metabolism and steroid synthesis, becoming an interesting study candidate for BP interaction.

Purpose: We aimed to test whether the *CYP1A1* variants found in the BP patients negatively affected its function. Moreover, we wondered if the BPs could have a negative effect on CYP1A1 activity and if so, if this effect could be exacerbated by any of the variants.

Methods: We cloned cDNAs bearing each of the *CYP1A1* variants found in AFF patients (p.Arg98Trp. p.Arg136His, p.Ser216Cys and p. Val409Ile) and the wild type form, transfected them into SAOS-2 cells

and measured CYP1A1 activity in cell extracts. We compared the effect of commonly used BPs (Alendronate, Zoledronic Acid and Risedronate) on the enzymatic activity of the CYP1A1 variants.

Results: We observed that RNA expression remained stable for all the variants. Interestingly, two of them (p.Arg98Trp and p.Arg136His) resulted in a severe reduction of enzymatic activity compared to the wild type ($p<0,05$). BP treatments negatively affected all CYP1A1 variants' activity as well as the wild type form ($p<0,05$). However, no differences were found in the BP effect among the different CYP1A1 forms.

Conclusion(s): We demonstrate that the p.Arg98Trp and p.Arg136His variants found in the AFF patients have a significant negative effect on CYP1A1 enzymatic activity. Moreover, BPs decrease CYP1A1 activity but no specific interaction with any CYP1A1 variants has been found. Our results raise the hypothesis that an additive effect between CYP1A1 heterozygous mutations p.Arg98Trp and p.Arg136His and long term BP exposure generates susceptibility to AFF.

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P090

Transcriptomic analysis in the TgRANKL mouse model of osteoporosis reveals miRNAs as potent regulators of bone remodeling

Elisavet Ioannidou[a], Vagelis Rinotas[a,b], Lenka Plestilova[c], Astrid Jüngel[c], Trias Thireou[b], Eleni Douni[a,b]
[a]*Biomedical Sciences Research Center "Alexander Fleming", Institute for Bioinnovation, Athens, Greece*
[b]*Agricultural University of Athens, Department of Biotechnology, Athens, Greece*
[c]*University Hospital Zürich, Center of Experimental Rheumatology, Zurich, Switzerland*

Background/Introduction: Osteoporosis is a multifactorial disease characterized by bone loss, bone fragility and increased risk of fractures. Our lab has established a genetic mouse model of osteoporosis by overexpression of human RANKL in transgenic mice (TgRANKL) for the elucidation of the molecular basis in bone resorption.

Purpose: The purpose of the current study was the identification of differentially expressed genes with potential clinical value in osteoporotic TgRANKL mice.

Methods: We conducted RNA-seq for mRNAs and miRNAs in flushed femurs from TgRANKL and wild type (WT) littermates. Deregulated genes were analyzed with bioinformatics tools, and were confirmed experimentally with qPCR.

Results: As regards mRNA analysis we identified in total 2747 differentially expressed (DE) mRNAs ($|\log_2\text{FoldChange}| > 1$, p-adjusted < 0.05), including 959 upregulated and 1788 downregulated in TgRANKL femurs compared to WT. Enrichment analysis showed that upregulated mRNAs were clustered in biological processes related to bone remodeling, mineral absorption, ossification, protein digestion and absorption, cytokine-receptor interaction, and lysosome, whereas the downregulated mRNAs were clustered in processes related to mitochondrial activity and muscle function. Selected genes from each category were validated using qPCR.

As concerns miRNA analysis, we identified 63 DE miRNAs ($|\log_2\text{FoldChange}| > 1$, p-adjusted < 0.05), containing 33 upregulated, and 30 downregulated in TgRANKL femurs compared to WT. Among DE miRNAs, 12 have already been reported in osteoporosis (i.e. miR-133a and miR-21a), 29 miRNAs were sparsely studied in skeletal diseases, while 22 remain uncharacterized. Correlation analysis showed that plenty of DE mRNAs were negatively correlated with the DE miRNAs, while pairs of the negatively correlated miRNA-mRNA were predicted as possible interactors through miRWalk target analysis. Expression of selected miRNA-mRNA pairs are currently validated through qPCR.

Conclusion(s): Conclusively, the differentially expressed genes revealed in this study may provide the basis for the identification of novel regulatory mechanisms and the establishment of novel biomarkers in osteoporosis.

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P091

From the voice of patients and caregivers: Burden of illness in infantile onset ABCC6 and ENPP1 deficiency (GACI and ARHR2)

Christine O'Brien[a], Beth Leiro[b], Gus Khursigara[c], Catherine Nester[c], Pedro Huertas[c]
[a]*GACI Global, Co-President, Boston, United States*
[b]*Origin Biosciences, N/a, Chapel Hill, United States*
[c]*Inozyme Pharma- Inc., Medical Affairs, Boston, United States*

Background/Introduction: Patients with infantile onset ABCC6 and ENPP1 deficiencies experience multisystem dysfunction due to pathological mineralization.

Purpose: To characterize burden of disease from a patient perspective.

Methods: Primary qualitative patient reported outcomes (PRO) research under IRB approval was performed to determine disease burden in patients with infantile onset ABCC6 or ENPP1 deficiency of all ages.

Results: Thirty-eight respondents from 9 countries with ABCC6 deficiency (N=6) or ENPP1 [infantile (n=12), pediatric (n=13) or adult (n=7)] were assessed. Parents of patients responded for those <18 years (n=31). Eleven were deceased, ten within the first 12 months of life. The most common symptoms for ABCC6 deficiency were cardiac (67%), gastrointestinal (83%) and growth and development (83%); and for ENPP1 deficiency were (adult, pediatric), cardiac (86%, 85%), bone and joint pain (100%, 85%) and mobility (86%, 85%). Most common burdens (unaided) were fear of unknown (53%) and cardiac (33%) in ABCC6 deficiency; and cardiac (50%) and difficulty with hospital experience (42%) for infantile ENPP1 deficiency, self-care related to management (50%) and hearing loss (42%) for pediatric ENPP1 deficiency, and bone and joint pain (71%) and mobility (57%) for adults with ENPP1 deficiency. Dental issues were noted in 24% of respondents.

Conclusion(s): The burden of disease in different age groups reflects the disease evolution. 50% early mortality due to cardiac complications is reflected in the burden of hospital experience and cardiac issues to parents of infants. Complex medical management poses a large burden for parents of pediatric patients. In adults, the cumulative impact of the cardiovascular and skeletal complications is reflected in the reports of pain, mobility impairment, and fatigue highlighting the chronic severe nature of the disorder. This study demonstrates that ENPP1 deficiency is a chronic and highly morbid disease for patients of all ages and is reflected in physical, emotional, and social burdens noted across the age continuum.

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P092

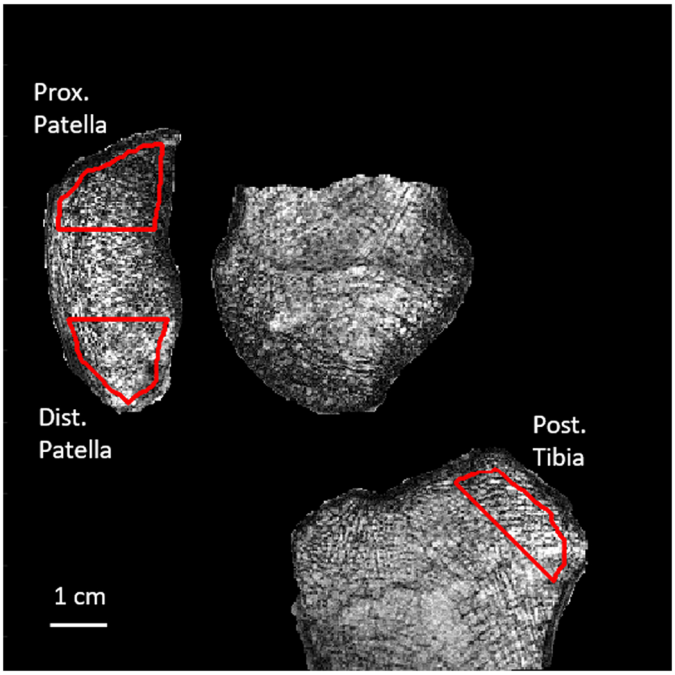
In vivo bone microarchitecture analysis in a psoriatic arthritic patient before and after anti-TNFα treatment

Enrico Soldati[a], Lucas Escoffier[b], Sophie Gabriel[c], Jean Pierre Mattei[b], Serge Cammilleri[c], David Bendahan[a], Sandrine Guis[a]
[a]*Aix-marseille University, CNRS- CRMBM-CEMEREM- umr CNRS 7339, Marseille, France*
[b]*Aix-marseille University, Service de Rhumatologie- AP-HM-, Marseille, France*
[c]*Aix-marseille University, Institut Fresnel- Service de Médecine Nucléaire- AP-HM- CNRS UMR 7249, Marseille, France*

Background/Introduction: Psoriatic arthritis (PA) is an inflammatory rheumatism, mediated in part by TNFα and associated with bone loss. Anti-TNFα treatment should inhibit this phenomenon and reduce the systemic bone loss. Ultra-high field MRI (UHF MRI) may be used to quantify bone microarchitecture (BM) in-vivo.

Purpose: Our purpose was to quantify BM using UHF MRI in a PA patient and to follow up changes related to anti-TNFα treatment.

Methods: A non-treated PA patient with knee arthritis and 7 gender-matched controls were scanned using a gradient re-echo sequence at UHF MRI. After a year of Adalimumab treatment, the patient underwent a second UHF MRI. A PET-FNa imaging was performed before and after treatment to identify and localize the abnormal metabolic areas. BM was characterized using typical morphological parameters (bone volume fraction and trabecular thickness, spacing and number) quantified in 3 regions of interest (12 analyzed characteristics in total) corresponding to tendon insertion: proximal and distal patellar, and posterior tibial. Statistical analysis was assessed performing Student T-tests.



Results: PET-FNa recorded before the treatment illustrated hypermetabolic areas which resumed after the treatment while the patient was in remission. Before treatment, the BM parameters were statistically different ($p>0.01$) from controls in 7/12 characteristics with differences reaching up to 38%. After treatment, BM parameters remined statistically different ($p>0.01$) in 3 out of 12 characteristics, trabecular spacing and number of proximal tibia and trabecular thickness of the distal patella.

		Healthy	P. before treatment	P. after treatment
Proximal Patella	BVF	0.393±0.008	0.339±0.018 *	0.401±0.010
	TbTh	0.254±0.013	0.254±0.022	0.266±0.014
	TbSp	0.364±0.032	0.477±0.058	0.365±0.008
	TbN	1.550±0.074	1.301±0.136	1.493±0.073
Distal Patella	BVF	0.355±0.035	0.222±0.064 *	0.328±0.027
	TbTh	0.261±0.010	0.250±0.015	0.285±0.004 *
	TbSp	0.469±0.117	0.651±0.057 *	0.532±0.064
	TbN	1.366±0.114	0.994±0.090 *	1.116±0.083
Posterior Tibia	BVF	0.381±0.009	0.307±0.016 *	0.335±0.018
	TbTh	0.258±0.008	0.260±0.009	0.267±0.012
	TbSp	0.426±0.060	0.594±0.012 *	0.570±0.016 *
	TbN	1.468±0.073	1.185±0.047 *	1.241±0.029 *

Conclusion(s): Our results illustrated knee microstructure alterations in a PA patient and a normalization after a year of treatment.The abnormalities initially observed were not only localized in the hypermetabolic regions identified by PET-FNa,suggesting that the bone loss was global and not related to inflammatory sites.Using UHF MRI, we highlighted and quantified in vivo BM anomalies in a patient with an inflammatory rheumatismtogether with the reversibility after one year of treatment.

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P093

Use of TNF-alpha inhibitors in rheumatoid arthritis and implications for the periodontal status: For the benefit of both?

Teun de Vries, Fatima Zamri
University of Amsterdam and VU University, Periodontology, Gustav Mahlerlaan, Netherlands

Background/Introduction: The inflammatory bone diseases rheumatoid arthritis (RA) and periodontitis show similarities in misbalances of cytokine levels, such as tumor necrosis factor-α (TNF-α). RA has been treated for two decades with TNF-α inhibitors which are effective by blocking TNF-α's destructive action.

Purpose: Since RA and periodontitis show similarities in high levels of TNF-α, the periodontal status of RA patients may improve with the use of anti-TNF-α therapy.

Methods: To assess this, a systematic review with special emphasis on duration of therapy was performed to evaluate the effect of anti-TNF-α treatment on the periodontal status of RA patients.

Results: Overall, studies showed an improvement in periodontal health with anti-TNF-α therapy, with $p<0.05$. When analyzed over time (6 weeks to 9 months), it became apparent that initial improvements concerned bleeding on probing (BOP) and gingival index (GI) after therapy duration of 6 weeks. Periodontitis parameters that improved after prolonged treatment were: probing pocket depth (PPD) after 3 months and clinical attachment level (CAL) after 6 months.

Conclusion(s): In conclusion, this systematic review reveals that anti-TNF-α treatment is therefore not only beneficial for rheumatic joints but also for the gums of rheumatoid arthritis patients. We propose that the sequential tissue recovery due to anti-TNF-α therapy progresses as follows: (1) block of diapedesis by lowering vessel permeability, (2) fewer leukocytes in the inflamed tissue, (3) reduced proteolytic activity and subsequent repair of collagen fiber functionality and normalization of osteoclast activity. Clinically, this could lead to a decrease in bleeding on probing and ultimately in an improved clinical attachment level.

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P094

Hip osteoarthritis in juvenile idiopathic arthritis is complication of the disease course or side effect of systemic glucocorticosteroid treatment?

Lubov Sorokina[a], Ilia Avrusin[a], Rinat Raupov[a], Sergey Khrypov[b], Mikhail Kostik[a]
[a]*St. Petersburg State Pediatric Medical University, hospital pediatric department, Saint Petersburg, Russia C.I.S.*
[b]*Saint-Petersburg clinical research-practical center for specialized healthcare oncology, surgical, Saint Petersburg, Russia C.I.S.*

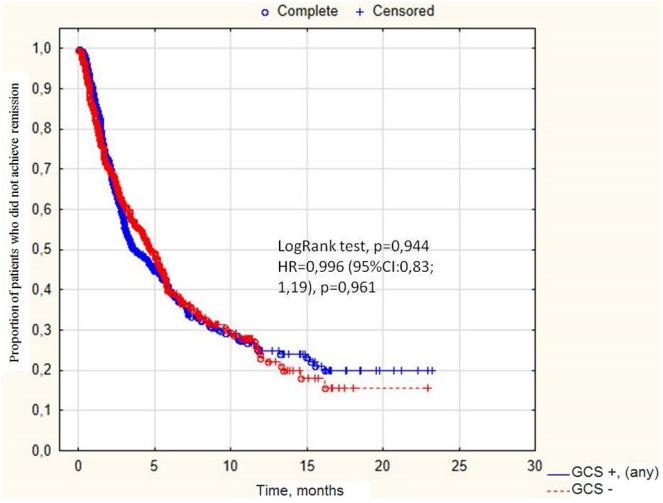
Background/Introduction: Glucocorticosteroid (GCS) treatment is still prescribed in systemic and non-systemic subtypes of juvenile

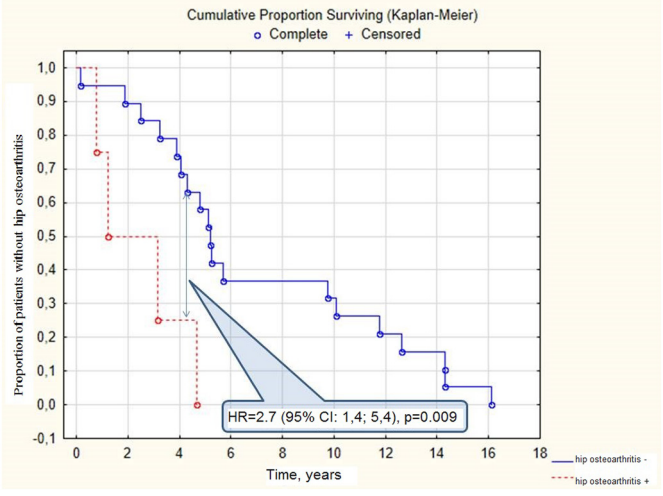
idiopathic arthritis (JIA) despite the limited indication for it use such as life-threatening course of systemic JIA.

Purpose: the evaluation of GCS treatment in JIA in previous decade, its efficacy, safety and correlation with irreversible hip changes.

Methods: Diagnosis JIA was made according to ILAR criteria. 753 JIA patients were included into the study during the period 01.01.2007-31.12.2016. There were 445 (59.1%) patients with experience of GCS treatment, including systemic GCS (n=287; 38.1%) and only intraarticular GCS injections (n=314 (41.7%). Systemic GCS included oral (n=152; 20.2%) and high-dose intravenous GCS administration (n=135; 18%). We evaluate the incidence of hip osteoarthritis and hip avascular necrosis development. The study protocol was not reviewed by the Ethics Committee as the authors worked with case histories.

Results: Children treated with GCS had earlier JIA onset age, higher inflammatory activity, more active joints and required of biologic frequently. The remission probability did not depend on GCS treatment (fig. 1). Hip osteoarthritis developed in 32 (4.3%) and 16 (2.1%) need total hip replacement (THR). GCS cumulative dose 2700 mg increased the risk of hip osteoarthritis (OR=2.7 [95%CI: 1.4; 5.4], p=0.009), fig.2. All patients except one who underwent for THR had experience of systemic GCS treatment and all of them had cumulative dose greater than 2700 mg.





Conclusion(s): Nowadays there is inappropriate high rate of GCS in JIA. Systemic GCS treatment is strongly associated with hip osteoarthritis and total hip replacement. We suppose that hip osteoarthritis with experience of systemic GCS treatment has avascular pathway and preventable. Systemic GCS did not show the benefits above non-GCS treatment in the remission achievement. Pediatric rheumatologist must avoid systemic GCS in non-systemic JIA patients excluding only life-threatening conditions. This work supported by the Russian Foundation for Basic Research (grant № 18-515-57001).

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P095

Effect of eriocitrin on the inflammatory disorders of human being: Therapeutic potential through data analysis of scientific research work

Kanika Patel[a], Dinesh Kumar Patel[b]
[a]*SHUATS, Department of Pharmaceutical Sciences, Allahabad, India*
[b]*Sam Higginbottom University of Agriculture- Technology and Sciences, Department of Pharmaceutical Sciences, Prayagraj, 211007- Uttar Pradesh, India*

Background/Introduction: Citrus limon is one of the common most plants of the traditional medicine which contains numerous pharmacologically bioactive compounds which prevents obesity and related complications. Eriocitrin is also called eriodictyol 7-rutinoside is a flavonoidal compound having better antioxidant potential and lipid-lowering activity in the high-fat diet induced rats. Inflammatory bowel disease (IBD) is inflammatory disorders of alimentary canal having symptoms of diarrhea, abdominal pain and fistula and can categorize into two distinct forms i.e. ulcerative colitis, and Crohn's disease. IBD can be generated due to microbial infections and environment factors which may affect quality of life.

Purpose: Anti-degenerative effect of eriocitrin alone and formulation have been known for their effectiveness on osteoarthritis damage.

Methods: Here in the present investigation, anti-inflammatory potential of eriocitrin have been investigated through data analysis of various scientific research work of the literature source. Role of antioxidant capacity, IL-6, MCP-1 and C-reactive protein in the anti-inflammatory potential of eriocitrin have been evaluated through scientific research data analysis of various literature source. Molecular data have been correlated with their pharmacological activity to predict better mechanism of eriocitrin in the treatment of inflammatory disorders.

Results: Effect of eriocitrin on the experimental animals of colitis have been investigated through literature data analysis of scientific research in the present investigation and revealed the significant effect on inflammatory cytokines. Eriocitrin has been investigated for their anti-oxidative stress and systemic anti-inflammatory potential on high-fat diet in C57BL/6J mice through another scientific research work of the literature. Scientific data analysis of the literature source revealed the anti-inflammatory potential due to higher antioxidant level and inhibitory potential on IL-6, MCP-1 and TBARS levels.

Conclusion(s): Literature data analysis of various scientific research works clearly revealed the effectiveness of eriocitrin against colitis and had protective activity on various form of inflammation and oxidative stress.

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P096

Anti-inflammatory activity of bavachinin against various forms of inflammatory disorders including asthmatic inflammation: Importance of natural medicine in the current scientific research

Kanika Patel[a], Dinesh Kumar Patel[b]
[a]SHUATS, Department of Pharmaceutical Sciences, Allahabad, India
[b]Sam Higginbottom University of Agriculture- Technology and Sciences, Department of Pharmaceutical Sciences, Prayagraj, India

Background/Introduction: Asthmatic inflammation is one of the major causes of death in the medical field as it is mediated through type 2 helper T cell (Th2) cytokine response. Flavonoidal compound including bavachinin have been isolated from *Psoralea corylifolia*, a herbs mainly known for their effectiveness on various form of skin disorders of Human being.

Purpose: Inflammation is the outcome of the human body defense system against various form of foreign threats entered in our body. Inflammation plays an important role in the development of various forms of human disorders in the medicine and other health sectors.

Methods: In order to know the anti-inflammatory potential of bavachinin in the medicine, numerous scientific research works data have collected and been analyzed in the present investigation to know the biological importance and therapeutic benefit of bavachinin against various forms of inflammatory disorders including Asthmatic inflammation. Pharmacological activities of bavachinin for their anti-inflammatory activity have been analyzed in the present investigation through literature data analysis of various scientific research works. Therapeutic role of different inflammatory mediators such as IL-4, IL-5 and IL-13 in the inflammatory disorders were also studied through literature data analysis of various scientific research work.

Results: From the literature data analysis of various scientific research works, it was found that bavachinin had significant biological potential in the medicine as it have positive role on Th2 cytokine production, IL-4, IL-5 and IL-13. In another scientific research work of the literature it showed effectiveness in the ovalbumin (OVA)-sensitized asthma model of animal. However literature data analysis of other scientific research works in the literature it also signified the biological importance bavachinin on IL-6-induced STAT3 promoter activity in Hep3B cells.

Conclusion(s): Literature data analysis of various scientific research works in the scientific field revealed the biological importance of bavachinin against different inflammatory disorders including Asthmatic inflammation.

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P097

Biological potential of sinensetin in the medicine for the treatment of various forms of inflammatory disorders: Therapeutic benefit in the medicine

Dinesh Kumar Patel
Sam Higginbottom University of Agriculture- Technology and Sciences, Faculty of Health Sciences, Prayagraj, India

Background/Introduction: Colitis is a disorder of intestinal epithelial barrier dysfunction which plays an important role in the development of various forms of intestinal inflammatory disorders of human being. Sinensetin is an important flavonoidal class phytochemical found to be present in the tangerine peel and other citrus fruits.

Purpose: To understand the therapeutic benefit of sinensetin in the medicine for the treatment of various form of inflammatory disorders.

Methods: In order to understand the therapeutic benefit of herbal drugs in the medicine for the treatment of various form of inflammatory disorders, here in the present investigation, biological importance of sinensetin on colitis has been studied in the present investigation through scientific data analysis of various research works of the literature. Further in order to know the effectiveness of sinensetin on various animal model of colitis, numerous scientific researches have been collected and analyzed through literature databases in the medicine and other allied health sectors.

Results: Scientific data analysis of various research works of the literature databases revealed the biological importance of sinensetin in the medicine for the treatment of various form of inflammatory disorders including colitis. Scientific data analysis revealed the biological importance of sinensetin in the medicine as it increase intestinal permeability and promoted epithelial cell autophagy. Further analysis of sinensetin through various scientific databases revealed significant effect on intestinal barrier dysfunction in colitis.

Conclusion(s): Scientific data analysis revealed the biological potential of sinensetin in the medicine for the treatment of various form of inflammatory disorders including colitis.

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P098

The anti-inflammatory potential of probiotics on skeletal diseases: Systematic review and meta-analysis of randomised controlled trials

Jenny Michael[a], Maria Moiseos[a], Nuno Ferreira[b], Antonia Sophocleous[a]
[a]European University Cyprus, Life Sciences Department, Nicosia, Cyprus
[b]University of Nicosia, Social Sciences Department, Nicosia, Cyprus

Background/Introduction: Interest on the therapeutic effect of intestinal microbiome and probiotics on various immune-mediated diseases has recently increased.

Purpose: To systematically identify and critically evaluate randomised controlled trials (RCTs) investigating the efficacy of probiotics as anti-inflammatory therapies for extraintestinal, skeletal-related diseases (e.g. rheumatoid arthritis, osteoarthritis, and osteoporosis).

Methods: A systematic search using the databases Medline, Embase and Web of Science was performed to identify randomised controlled trials investigating the therapeutic effect of probiotics on skeletal-related diseases. Risk of bias was assessed using Cochrane Collaboration's tool. Preliminary analysis of outcome measures included mean changes in serum levels of C-reactive protein (CRP), Tumour necrosis factor a (TNFa), and Interleukin-1b (IL-1b), following a period of probiotic treatment.

Results: A total of 17 studies were included in a qualitative synthesis, 13 of which were suitable for meta-analysis using the Review Manager Software. Eight of these studies focused on rheumatoid arthritis patients, one on osteoporosis, two on osteoarthritis, one on spondyloarthritis and a final study on Juvenile idiopathic arthritis. Preliminary results showed that after an 8-week to 1-year intervention period, probiotics supplementation significantly reduced serum CRP compared with placebo in all diseases collectively (standardised mean difference -1.25, all 95% confidence intervals -1.45, -1.05). Similarly, compared with placebo, probiotic supplementation resulted in a significant decrease in the pro-inflammatory cytokines TNFa and IL-1b (TNFa standardised mean difference -0.88, 95% CI -1.19, -0.58; IL-1b mean difference -0.71, 95% CI -1.01, -0.42). Similar effects were found when looking at each disease separately.

Conclusion(s): Although most of the studies identified involved rheumatoid arthritis (RA) patients, our results suggest that probiotic supplementation has beneficial effects on serum CRP, TNFa and IL-1b in a

spectrum of different bone diseases including rheumatoid arthritis, osteoporosis, osteoarthritis, spondyloarthritis and Juvenile idiopathic arthritis.

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P100

Human umbilical cord mesenchymal stem cells inhibited the aseptic joint loosening caused by wear particles in mice

Yue Ding, Li Shixun, Wu Chuangran
SYSU, orthopedics, Guangzhou, China

Background/Introduction: For patients who took joint replacement, one of the complications, aseptic joint loosening, could cause a high risk of revision surgery. But there were few treatments that could stop the osteolysis. Studies have shown that MSCs have the ability of homing and differentiating, and also have highly effective immune regulation and anti-inflammatory effects. However, few studies had focused on the stem cells in preventing the occurrence and development of aseptic loosening.

Purpose: In this research, we aimed to clarify whether human umbilical cord mesenchymal stem cells could inhibited the aseptic joint loosening caused by wear particles.

Methods: A Cranial osteolysis mice model was established on mice to examine the effect of hUC-MSCs on the Titanium particles injection area through micro-CT. The amount of stem cells injected was 2×10^5 cells. One week later, the mouse Cranial were obtained for micro-CT scan, and then stained with HE analysis immunohistochemical analysis of TNF-α, CD68, CCL3 and Il-1β.

Results: All mice were free of fever and other adverse reactions, and there was no death occurred. Titanium particles caused the osteolysis at the mice cranial, while local injection of hUC-MSCs did inhibit the cranial osteolysis, with a lower BV/TV and a higher porosity. Immunohistochemical results suggested that the expression of TNF-α, CD68, CCL3 and Il-1β in the cranial in Titanium particles mice increased significantly, but was significantly reduced in mice injected with hUC-MSCs. The inhibited CD68 expression indicated that the number of macrophage was lower, which might be a result of the inhibition of CCL3.

Conclusion(s): According to the studies above, HUC-MSCs treatment of mouse cranial osteolysis model can significantly reduce osteolysis, inhibit macrophage recruitment and alleviate inflammatory response. It may become a promising treatment of aseptic joint loosening.

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P101

Ex vivo biomarker profiling identifies Oncostatin-M as a spine osteoarthritis-specific target

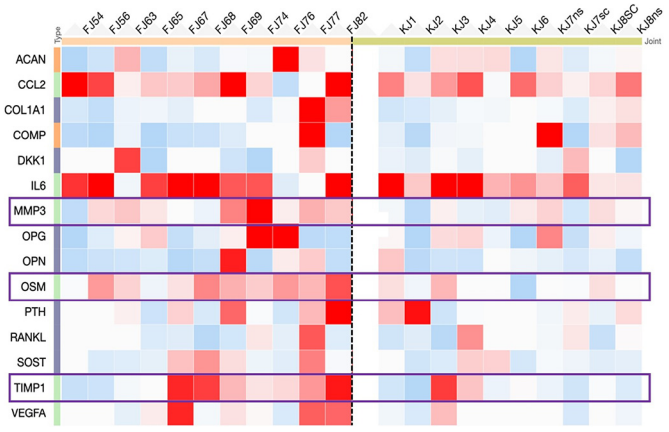
Olivier Roessinger[a], Marc Blanchard[a], Cordula Netzer[b], Timo Ecker[c], Thomas Hügle[a], Jeroen Geurts[a]
[a]*Lausanne University Hospital, Rheumatology, Lausanne, Switzerland*
[b]*University Hospital of Basel, Spine Surgery, Basel, Switzerland*
[c]*Lausanne University Hospital, Spine Surgery, Lausanne, Switzerland*

Background/Introduction: Disease heterogeneity, both clinically and molecularly, has been a major hurdle in the development of efficacious disease-modifiying osteoarthritis drugs (DMOADs). Disease heterogeneity does not only occur within, but also between joint types. However, robust data on joint-specific pathomechanisms of osteoarthritis (OA) are still lacking.

Purpose: Here, we performed *ex vivo* biomarker profiling of human osteochondral tissue of knee and spine OA to identify joint-specific pathomechanisms and DMOAD treatment responses.

Methods: Facet joint and tibial plateaus were obtained from patients undergoing lumbar spinal fusion (n=10, mean age 72.8) and total joint arthroplasty (n=8, mean age 73.0) respectively. Osteochondral specimens were randomly assigned to treatment groups: control (DMSO), inflammation (1 μg/mL LPS) or inflammation + DMOAD (TGF-beta type I receptor inhibitor,10 μM SB-505124). Explant culture was conducted for one week and biomarkers of bone metabolism (Pro-Col-Ia, SOST, OPG, OPN, DKK1, PTH, RANKL), inflammation (MCP-1, IL-6, MMP3, OSM, TIMP1, VEGFA) and cartilage metabolism (ACAN, COMP) were determined by ELISA.

Results: Clusteranalyses revealed that LPS stimulation increased IL-6 and MCP-1 secretion by both facet joint (FJ) and knee joint (KJ) tissues. Interestingly, Oncostatin-M (OSM) and its downstream mediators MMP3 and TIMP1 were increased in the majority of FJ, but not KJ specimens (***Figure***). Statistical analyses corroborated increased OSM, MMP3 and TIMP1 levels in a spine-specific fashion. DMOAD treatment led to a drastic reduction of Pro-Collagen-Ia and IL-6 secretion in both spine and knee OA specimens. Interestingly, OSM, TIMP1 and MMP3 levels were reduced in FJ specimens only.



Conclusion(s): Oncostatin-M expression and signaling was uncovered as specific pathomechanism of spine OA. Known to be predominantly expressed by macrophages and immune cells, OSM may be an important osteoimmunological mediator of tissue damage and remodeling in spine, but not knee OA. This study also highlights the value of ex vivo human tissue models for OA phenotyping and preclinical evaluation of DMOADs.

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P106

Vitamin D supplementation reduces the risk of fall in the vitamin D deficient elderly: An updated systematic review and meta-analysis

Yali Ling, Zhongjian Xie
The Second Xiangya Hospital of Central South University, Department of Metabolism and Endocrinology, Changsha, Hunan, China

Background/Introduction: Vitamin D supplementation has been widely recommended to prevent falls. However, considerable controversy exists regarding the association of such supplementation and fall risk.

Purpose: Previous meta-analyses yielded inconsistent results because of differences in the baseline of 25(OH)D and dose of vitamin D and use of vitamin D or in combination with calcium in different studies.Therefore, an updated and comprehensive meta-analysis is warranted.

Methods: We systematically searched several literature databases including PubMed and Embase database from inception to September 2020. RCTs reporting the effect of vitamin D supplementation alone or with calcium on fall incidence were selected from studies. Qualitative and quantitative information was extracted; the random-effects model was conducted to pool the data for fall.

Results: Of the citations retrieved, 31 eligible studies involving 57867 participants met inclusion criteria. A total of 21 RCTs of vitamin D alone and 10 RCTs of vitamin D plus calcium were included in the meta-analysis. A meta-analysis of 21 RCTs (51984 participants) of vitamin D supplementation alone did not show a reduced risk of falls (RR 1.00, 95% CI 0.95 to 1.05) compared to placebo or no treatment. Subgroup analyses showed that the baseline of serum 25(OH)D concentration less than 50 nmol/l resulted in a reduction of fall risk (RR 0.77, 95% CI 0.61 to 0.98). The meta-analysis of 10 RCTs (5883 participants) of combined supplementation of vitamin D and calcium showed a 12% reduction in the risk of fall (RR 0.88, 95% CI 0.80 to 0.97).

Conclusion(s): The combination of vitamin D and calcium have beneficial effects on prevention falls in old adults. Although vitamin D supplementation alone has no effect on fall risk in old adults with 25 (OH)D levels higher than 50 nmol/L, vitamin D supplementation alone does have a benefit on prevention falls in old adults with 25(OH)D levels lower than 50 nmol/L.

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P108

Quality of life changes in women with osteoporotic vertebral fractures and possibility of its improvement using new complex of physical therapy including mechanotheraputic technologies

Larisa Marchenkova, Vasileva Valeria
National Medical Research Center of Rehabilitation and Balneology-Rehabilitation department for somatic patients, Rehabilitation department for somatic patients, Moscow, Russia C.I.S.

Background/Introduction: New comprehensive rehabilitation programs including physical exercise and mechanotherapeutic technologies is promising method of quality of life (QOL) improving in patients with vertebral fractures (VFs) associated with osteoporosis.

Purpose: To assess QOL in patients with osteoporotic VFs and evaluate effect of new complex of physical rehabilitation including mechanotherapy on QOL of those patients.

Methods: At the 1st stage study group was comprised of 60 women 40-80 years old with osteoporotic VFs. The comparison group (n=60) was formed from patients with osteoporosis but without any fracture. 2nd stage was carried out in the form of a prospective controlled open study. 120 patients with osteoporotic VFs were randomized in two groups. The intervention group (group 1, n=60) received a new complex of physical therapy including back muscle training with mechanical loads #10; sensorimotor training on double unstable platform #10; kinesohydrotherapy in a pool #15; physical exercises in a gym #10. Group 2 was prescribed only physical exercises in a gym #15. QOL was assessed in all patients with QUALEFFO-41 scale at baseline, at 21st day at the end of rehabilitation and at 70th day as follow-up.

Results: In patients with VFs a significant decrease in main QOL domains such as severity of pain, daily living activity, mobility, mental state, general health and general QUALEFFO-41 scale was revealed ($p<0.05$ vs comparison group). Administration of a new physical rehabilitation complex resulted in pain reduction and improvement of such QOL aspects as house jobs, mobility and mental state ($p<0.05$ at 21st day day vs baseline). Therapy effect on pain syndrome, daily living activity, mobility and overall QOL remains for at least 4 weeks after the rehabilitation course ($p<0.05$ at 70th day vs baseline).

Conclusion(s): New physical therapy complex including mechanotherapeutic technologies can be recommended for rehabilitation of patients with osteoporotic VFs to increase QOL and to reduce back pain.

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P109

Elastofibroma dorsi. An entity to consider

Blanca Varas de Dios
Santa Cristina Universitary Hospital, Rheumatology, madrid, Spain

Background/Introduction: Painful shoulder is a common pathology. Osteotendinous etiology is the most common but we must consider others.

Purpose: A 57-year-old woman with persistent pain in right shoulder for 4 months.

Methods: She refers to joint clicking with mobilization for years. Associated with occasional pain and clicking in the left shoulder. On examination, a non-painful infrascapular mass and clicking with abduction and external rotation. No joint limitation but pain in all ranges of motion. In the joint ultrasound there is an image compatible with a soft tissue tumor without signs of malignancy. The MRI confirms the existence of the lesion affecting the connective tissue on the lateral aspect of the right rib cage between the ribs and the serratus, without bone or pleural infiltration, compatible with elastofibroma dorsi (ED). We found a smaller left supraclavicular ED.

Results: ED is an underdiagnosed unusual lesion with an accidental diagnosis. It´s a rare benign fibroelastic tissue tumor. It´s more common in women after the fifth decade. The most common location is the subscapular region. Also described in the suprascapular, deltoid, ischium, olecranon and feet level. It predominates on the right side, however, there are bilateral or synchronous cases.The diagnosis is clinical. On physical examination, the lesion is usually well circumscribed without adhering to the overlying skin. It´s difficult to delineate with respect to neighboring structures. The tumor is moveable, becoming palpable and more painful. Its most frequent location is anterior to the scapula, between 6-8 rib. Ultrasound, CT and MRI are the most used complementary examinations to confirm the diagnosis. The differential diagnosis includes osteotendinous pathology, and other benign tumors and cancer. Treatment is conservative, excision only being performed in highly symptomatic processes.

Conclusion(s): The ED must be known in order to differentiate it from other entities. We can avoid invasive techniques such as joint infiltrations or excision.

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P110

Development of a virtual reality-based training for the elderly with increased fracture risk to prevent falls and improve their balance

Felix Nikolai Schmidt[a], Stefan Gerlach[b], Malte Issleib[c], Alexander Schlaefer[b], Björn Busse[a]
[a]*University Medical Center Hamburg-Eppendorf, Department of Osteology and Biomechanics, Hamburg, Germany*
[b]*Hamburg University of Technology, Institute of Medical Technology and Intelligent Systems, Hamburg, Germany*
[c]*University Medical Center Hamburg-Eppendorf, Department of Anesthesiology, Hamburg, Germany*

Background/Introduction: Prevention of falls accompanied by severe injuries such as fracture is of high importance for our aging society. Beyond muscle function, bone quantity and quality-indices, in particular body-control and -coordination are considered to have major impact on preventing fractures by minimizing fall-events themselves.

Purpose: Using virtual reality (VR) strategies to improve clinical parameters associated with reduced fracture risk. Recent reports indicate that specific training regimes using VR in elderly patients may lead to both mood improvement and significant advantages in balance and body control. Thus, it is becoming apparent that VR offers enormous potential for individualized, patient-centered medicine. Especially adressing the risk of shortage of critical medical supply for patients in rural areas with age-associated musculoskeletal conditions.

Methods: A Hololens-2 headsets is used to induce and monitor patient movements. The focus is on improving balance, body control and coordination in order to reduce the risk of falls. To this end, a virtual downhill skiing scenario was implemented. Patients see a target trajectory and control their motion by leaning forward, backward, or sideways to adjust speed and to steer. The course is defined such that patients have to initiate repeated, smooth body motion when following the trajectory.

Results: The software framework has been implemented and evaluated on healthy subjects. Generally, wearing the headset is tolerated well and interaction with software and motion-control was quickly learned. Active motion of the trunk and lower limbs was observed.

Conclusion(s): A VR-environment for musculoskeletal training of patients is under development. First tests indicate suitability of the system. Furthermore, it is extended to adapt to individual patient motions, to monitor and evaluate their motions with respect to improved body-balance and -control. The data will be linked to clinical data from DXA, HR-pQCT, gait and posture-analyses to analyze the musculoskeletal changes identifying the efficacy of VR in increased fracture risk cohorts.

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P112

Risk of sarcopoenia and prevention of disability in post COVID 19 elderly patients

Ferdinando D'Amico[a,b], D'Amico Rossella[c]
[a]*Hospital of Patti Messina, Department of Geriatrics, Patti Messina, Italy*
[b]*School of Medicine University of Messina, Geriatrics, Patti Messina, Italy*
[c]*IRCCS Oasi Maria SS Troina, Medical Rehabilitation, Troina, Italy*

Background/Introduction: It is possible to improve significant activities in elderly patients by creating a link between physical ability and quality of life.

Purpose: The study evaluates the effects of combined drug treatment and occupational therapy in the basic-instrumental activities of daily life, in physical ability and in the risk for sarcopoenia in post COVID 19 elderly subjects. 7 post COVID 19 elderly people (M 3, F 4, mean age 82 + 6) hosted in an Extended Care Unit were included in the study.

Methods: The design included tests performed before and after follow-up: 1) Mental State Examination (MMSE); 2) Geriatric Depression Scale (GDS); 3) Activities of Daily Living (ADL);4) Instrumental Activities of Daily Living (IADL); 5) Short Physical Performance Battery (SPPB). The subjects of the study showed: 1)Mean MMSE score was 23.7± 8.3; 2) Mean GDS score was 13±1.8. In the group 3 subjects showed an ADL score <3, 6 had IADL <4. All subjects were specifically treated pharmacologically for comorbidities. Through SPPB evaluation we detected a mean score of 6 in physical ability. To improve ADL and IADL a 6-day-per-week occupational therapy programme was introduced. This focused on teaching patients how to compensate and adapt either physically and socially.Presence or absence of cooperation in ADL is strongly linked to the depression level.

Results: Before and after a 2-month follow-up we detected: 1) GDS score 13±1.8 vs score 8±1.7 ($p<0.01$); 2) ADL 3/6 score vs 5/6 score ($p<0.01$); 3) IADL 4/8 score vs 6/8 score ($p<0.01$); 4) SPPB score 6 vs 8 ($p<0.01$).

Conclusion(s): The combination of drug treatment and occupational therapy in post COVID 19 patients showed an improvement both in ADL and IADL. We've also linked the increased physical ability to the risk reduction of sarcopoenia. The Occupational Therapist approach was customized in order to make the patients more self-assured and independent.

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P113

Subchondral vascular modifications for hydraulic pressure load transmission and osteoarthritis

Michael Beverly[a], Eleanor Vickers[b], David Murray[b]
[a]*University of Oxford, Botnar Research Centre, Oxford, United Kingdom*
[b]*University of Oxford, Botnar Research Centre- NDORMS- Nuffield Orthopaedic Centre, Oxford, United Kingdom*

Background/Introduction: With loading very high subchondral intraosseous pressure (IOP) occurs. Load is transferred partly by hydraulic pressure. There are previously undescribed subchondral vascular marks on MRI scans, reduced in osteoarthritis.

Purpose: We looked for structures that support load transmission by hydraulic pressure.

Methods: Normal and osteoarthritic upper tibial bone in the transverse subchondral plane was examined histologically.

Results: Radiating vessels running below the articular surface in the subchondral plane were found similar to the marks seen on MRI scans. The vessels were absent in osteoarthritic bone. Where the vessels penetrate the cortex near the joint margin there are complex choke-valve like distortions.

Conclusion(s): We confirm the presence of previously undescribed vessels running in the subchondral plane, consistent with the marks seen on MRI scans. As the vessels approach the cortical margin, complex distortions exist which may be choke-valves. With a raised surrounding IOP, they would close to prevent turbulent high-pressure flow in and out of the subchondral bone. Osteoarthritic bone had none of the longitudinal subchondral vessels or the subcortical choke valve morphological features. We suggest that osteoarthritis is associated with vasculo-mechanical failure.

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P114

Effect of resistance training on muscle texture of the thigh as measured by MRI

Oliver Chaudry[a], Mansour Ghasemikaram[a], Franz Jakob[b], Kemmler Wolfgang[a], Klaus Engelke[a]
[a]*Institute of Medical Physics, University of Erlangen-Nürnberg, Erlangen, Germany*
[b]*Bernhard-Heine-Center for Locomotion, University of Würzburg, Würzburg, Germany*

Background/Introduction: Sarcopenia is characterized by a progressive loss of skeletal muscle mass, which is infiltrated by adipose tissue (AT). Exercise can decrease the age related AT increase.

Purpose: To determine the effect of long-term high intensity resistance training (HIRT) on muscle (MT) and adipose tissue within the fascia lata.

Methods: Thirty-one community dwelling men (age > 72 years) were randomly assigned to a training group (T, n = 15) or an inactive control group (C, n = 16). MRI scans at mid-thigh were performed (3T, MAGNETOM Skyrafit, Siemens) before and after 16 months of training. Fat fraction (FF) and texture parameters of muscle tissue and of the intra-fascia volume (IF) were obtained from a 6 point Dixon sequence (length 10 cm, 160x160, 34 slices, 1.5x1.5x3.0 mm³). Parameters included global and local inhomogeneity, degree of anisotropy and compactness. Significance of longitudinal changes and between-group differences were determined by t-tests for parameters that did not correlate higher than R^2>0.7 with FF.

Results: Table 1 shows base line (BL) values, absolute changes (Δ) and significance of longitudinal changes for both groups. Training effects were significant with exception of the degree of anisotropy (p=0.07).

Parameter	Group	BL	Δ	p
FF IF [%] **	C	15.3 (4.1)	1.2 (0.78)	<0.01
	T	17.1 (5.6)	0.06 (1.0)	0.8
Local Inhom. IF **	C	48.0 (9.0)	1.8 (2.0)	<0.01
	T	50.8 (9.8)	–0.42 (2.3)	0.49
Degree Aniso. IF	C	1.56 (0.1)	0.001 (0.05)	0.9
	T	1.59 (0.1)	–0.04 (0.07)	0.04
FF MT [%] *	C	7.7 (1.9)	0.5 (0.2)	<0.01
	T	8.0 (2.2)	0.2 (0.4)	0.03
Local Inhom. MT *	C	33.2 (6.3)	0.9 (1.2)	<0.01
	T	34.1 (6.9)	0.03 (1.3)	0.9
Compactness MT *	C	0.02 (0.006)	–0.0009 (0.001)	<0.01
	T	0.02 (0.006)	0.00004 (0.001)	0.9

Conclusion(s): After 16 months, FF increased significantly more in the control than in the training group. After HIRT the AT distribution appeared to be smoother and more ordered than in the control group. While a final confirmation using a multivariate analysis is still pending our data indicated the power of a texture analysis to obtain additional information about muscle quality.

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P115

Skeletal muscle cell oxidative stress as a possible therapeutic target in a sarcopenia

Hideyuki Kinoshita[a], Sumihisa Orita[b], Kazuhide Inage[b], Seiji Ohtori[b]
[a]*Chiba Cancer Center, Orthopaedic Surgery, Chiba, Japan*
[b]*Chiba University, Orthopaedic Surgery, Chiba, Japan*

Background/Introduction: Oxidative stress has been reported to be involved in a number of pathologies, including musculoskeletal disorders. Its relationship with sarcopenia, one of the potential origins of lower back pain, however, is not yet fully understood.

Purpose: To elucidate the contribution of oxidative stress to muscle degeneration and the efficacy of antioxidant treatment for sarcopenia using an animal model of neurogenic sarcopenia.

Methods: Myoblast cell lines (C2C12) were treated with H2O2, an oxidative stress inducer, and N-acetyl-L-cysteine (NAC), an antioxidant. Apoptotic effects induced by oxidative stress and the antioxidant effects of NAC were assessed by western blotting, immunocytochemistry, and 3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide (MTT) cell viability assays. An animal model of sarcopenia was produced via axotomy of the sciatic nerves to induce muscle atrophy. Twenty-four male Sprague-Dawley rats were divided into sham, sham + NAC, axotomy, and axotomy + NAC groups. Rats were provided water only or water containing NAC (1g/L) for 4 weeks. The gastrocnemius muscle was isolated and stained with hematoxylin and eosin (H&E) 2 weeks after axotomy, from which muscle cells were harvested and protein extracted for evaluation.

Results: Mitogen-activated protein kinases (MAPKs) were significantly activated by H2O2 treatment in C2C12 cells, which was ameliorated by NAC pretreatment. Furthermore, H2O2 induced apoptosis and death of C2C12 cells, which was prevented by NAC pretreatment. The weight of the gastrocnemius muscle was reduced in the axotomy group, which was prevented by NAC administration. Lastly, although muscle specimens from the axotomy group showed greater reductions in muscle fiber, the oral administration of NAC significantly inhibited amyotrophy via antioxidant effects.

Conclusion(s): The current *in vitro* and *in vivo* study demonstrated the possible involvement of oxidative stress in sarcopenic pathology. NAC represents a potential anti-sarcopenic drug candidate, preventing amyotrophy and fatty degeneration.

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P117

Detection of low bone mineral density using radiofrequency echographic multi-spectrometry (REMS) in a pregnant woman with progressive scleroderma

Elena Kirilova[a], Nikola Kirilov[b]
[a]*UMBAL Dr. Georgi Stranski, Rheumatology, Pleven, Bulgaria*
[b]*UMBAL Dr. Georgi Stranski, Orthopedics and traumatology, Pleven, Bulgaria*

Background/Introduction: REMS is a non-ionizing innovative approach for the assessment of REMS-based bone mineral density (BMD) of the axial skeleton. In previous published studies it was envisaged to apply this technology for examination of the axial BMD in pregnant women. Systemic connective tissue diseases, such as scleroderma, are often treated with corticosteroids and other medications that cause bone loss.

Purpose: The aim of the current study is to assess REMS-based BMD, T- and Z-score values of both femora and lumbar spine in a pregnant woman with progressive scleroderma.

Methods: We present a clinical case of a 30-year-old Caucasian 12 weeks pregnant woman with a body mass index of 14.1 kg/m². She has been diagnosed for eight years with progressive scleroderma, myositis, Raynaud's phenomenon and digital ulcers. The patient was treated with D-penicillamine 300 mg daily and prednisolone 10 mg daily within five years before pregnancy and with cyclophosphamide pulse therapy within one year before pregnancy. REMS approach was used to assess REMS-based BMD and REMS-based Z-score values of the femoral neck, trochanter and total hip of both femora, as well as of the lumbar spine.

Results: T- and Z-score values were equal to -2.5 standard deviations (SD) on the both femora. BMD (g/cm²) was significantly reduced. These values of the lumbar spine were also outside the normal range. Although the spinal T-score remained under -2 SD, Z-score was “below the expected range for her age”.

Conclusion(s): The case demonstrates BMD, T- and Z-score values of both femora and lumbar spine in a pregnant woman with advanced progressive scleroderma assessed with the radiation-free REMS technology. This method could be very helpful for making decision about the treatment of pregnant women who are at risk of lower BMD due to concomitant diseases and/or medications that cause bone loss.

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P118

Forearm bone densitometry by radiography with a step-wedge phantom: A pilot study

Ling Wang[a], Xiaoguang Cheng[a], Pengjun Huang[a], Du Hui[b], Xinghua Yin[b], Tanuj Puri[c], Xieyuan Jiang[d], Klaus Engelke[e], Glen M. Blake[c]
[a]*Beijing Jishuitan Hospital, Radiology, Beijing, China*
[b]*Beijing Jishuitan Hospital, Department of Orthopedics, Beijing, China*
[c]*King's College London- St Thomas' Hospital, School of Biomedical Engineering & Imaging Sciences, London, United Kingdom*
[d]*Beijing Jishuitan Hospital, Department of Traumatic Orthopedics, Beijing, China*
[e]*FAU University Erlangen-Nürnberg and Universitätsklinikum Erlangen, Department of Medicine 3, Erlangen, Germany*

Background/Introduction: Radiographic absorptiometry (RA) is one of the earliest methods of bone densitometry and has been used to measure the phalanges and metacarpals where soft tissue attenuation is minimal.

Purpose: The aim of this study was to determine whether the technique can be adapted to correct for soft tissue and measure areal bone mineral density (aBMD) in the forearm.

Methods: Digital X-ray images of the left forearm and a step phantom were acquired in a training cohort of 30 subjects (21 F, 9 M) (mean age (SD): 62 (13) years) referred for routine dual-energy X-ray absorptiometry (DXA) examinations. Forearm DXA scans were performed on a GE-Lunar iDXA densitometer. Identical regions of interest (ROIs) in the proximal radius and ulna were measured on the X-ray and DXA images and a soft tissue ROI measured on X-ray images between the radius and ulna. X-ray measurements were expressed as equivalent step phantom thickness and used to estimate forearm aBMD using a linear equation calibrated against the GE-Lunar iDXA scans. Digital X-ray images were acquired in a second validation cohort of 30 subjects and the aBMD estimates compared with results of iDXA scans.

Results: Digital X-ray estimates of radius and ulna aBMD in the proximal forearm in the validation cohort showed a good correlation with iDXA measurements ($r = 0.795$). The Bland-Altman plot had a mean bias of -0.011 g/cm^2 and 95% limits of agreement -0.195 to +0.173 g/cm^2.

Conclusion(s): Digital X-ray estimates of proximal forearm aBMD corrected for soft tissue attenuation correlated with DXA measurements with correlation coefficients comparable to those seen for other peripheral bone densitometry technologies.

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P119

Comparison of methods to improve fracture risk assessment in Chinese diabetic postmenopausal women: A case-control study

Zhangxin Wen[a], Na Ding[a], Rong Chen[a], Qinyi Wang[a], Zhifeng Sheng[a], Hong Liu[b]
[a]*the Second Xiangya Hospital of Central South University, Department of Metabolism and Endocrinology, hunan, China*
[b]*the affiliated Zhuzhou Hospital Of Xiang Ya School Of Medicine of Central South University, Department of Metabolism Endocrinology, hunan, China*

Background/Introduction: Osteoporosis is a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue that leads to increased fracture risk, disability, financial burden, and mortality.The number of patients with osteoporosis in China is increasing with the aging population. Most widely used assessment instrument is the Fracture Risk Assessment Tool (FRAX®).Hpwever,the current FRAX formulation does not include T2DM among the risk factors, evidence suggests that the FRAX algorithm does not reflect the risk of fractures in T2DM patients correctly.There are several proposals to improve the FRAX performance for those with T2DM.

Purpose: This study compared the performance of three proposed Fracture Risk Assessment Tool (FRAX) alternatives to the current standard Chinese FRAX in predicting bone fracture risk in type 2 diabetic (T2DM) postmenopausal women, and to explore the optimal strategy to better predicted fracture risk in postmenopausal women with diabetes in China.

Methods: We recruited 434 patients from community-medical centers, 217 with T2DM and 217 without T2DM (non-T2DM). All participants completed self-reported questionnaires detailing their characteristics and risk factors. Bone mineral density (BMD) and spinal radiographs were evaluated. The China FRAX model calculated all scores. The area under the receiver operator characteristic curve (ROC-AUC) evaluated the sensitivity, specificity, and accuracy for predicting 10-year risk for major (MOF) and hip (OHF) osteoporotic fractures in T2DM patients.

Results: T2DM patients had higher BMD but lower average FRAX values than non-T2DM patients. The unadjusted FRAX ROC-AUC was 0.774, significantly smaller than that for 0.5-unit femoral neck T-score-adjusted FRAX (0.800; $p = 0.004$). Rheumatoid arthritis (RA; AUC = 0.810, $p = 0.033$) and T-score (AUC = 0.816, $p = 0.002$) adjustments significantly improved fracture prediction in T2DM patients.

Conclusion(s): Femoral neck T-score adjustment might be the preferred method for predicting MOF and OHF in Chinese diabetic postmenopausal women, while RA adjustment only effectively predicted HF risk.

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P120

Vfrac – a simple clinical tool that identifies older women with back pain at high risk of osteoporotic vertebral fractures

Emma Clark[a], Sarah Davis[b], Rachael Gooberman-Hill[a], Linda Hunt[a], Tarnjit Khera[a], Paskins Zoe[c], Peters Tim[a], Tobias Jon[a]
[a]*University of Bristol, Bristol Medical School, Bristol, United Kingdom*
[b]*University of Sheffield, School of Health & Related Research, Sheffield, United Kingdom*
[c]*Keele University, Arthritis Research UK Primary Care Centre, Keele, United Kingdom*

Background/Introduction: Osteoporotic vertebral fractures (OVFs) identify people at high risk of future fractures, but despite this, less than a third come to clinical attention. This is due to a variety of reasons including inadequate understanding of the clinical triggers necessary to refer high risk individuals for spinal radiographs.

Purpose: To develop a simple clinical tool to aid in deciding which older women with back pain should have a spinal radiograph.

Methods: 1634 women aged 65+ with back pain in the previous four months were recruited from primary care in two parts of the United Kingdom (NRES 18/WS/0061). Data were collected through self-completion questionnaires, physical examination and spinal radiographs. Exposure data included descriptions of back pain, traditional risk factors for osteoporosis, basic anthropometry and reported height loss. The outcome was the presence/absence of OVFs identified using the Algorithm-Based Qualitative method. Those with spinal metastases (n=3) or surgical fusion (n=30) were excluded. Logistic regression models identified independent predictors of OVFs. AUC for the final model was calculated. The choice of final cut-off for identification of which older women with back pain should have a spinal radiograph because of a high risk of fracture was based on a maximised sum of sensitivity and specificity.

Results: Mean age was 73.9 years (range 65.4 to 96.8), and 209 (12.8%) had OVFs. The final Vfrac model comprised 14 independent predictors of OVF, with an AUC of 0.802 (95%CI 0.764-0.840). Sensitivity was 72.4% and specificity 72.9%. Of those recommended for spinal radiographs on the basis of Vfrac, approximately one third had an OVF. Vfrac identified 93% of those with >1 OVF and two-thirds of those with one OVF. It identified 92% of those with severe OVFs and approximately two-thirds of those with mild/moderate OVFs.

Conclusion(s): The Vfrac clinical tool appears valid and now requires testing to establish real-world clinical and cost-effectiveness.

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P121

Osteoporosis care during the COVID-19 pandemic in the Netherlands: A national survey

Joséphine Peeters[a], Peter van den Berg[b], Joop P. van den Bergh[c], Marielle H. Emmelot-Vonk[d], Gijs de Klerk[e], Willem F. Lems[f], Elizabeth M. Winter[a], M. Carola Zillikens[g], Natasha M. Appelman-Dijkstra[a]

[a]*Leiden University Medical Centre, Center for Bone Quality- department of Internal Medicine- division of Endocrinology, Leiden, Netherlands*
[b]*Reinier de Graaf Gasthuis, Fracture Liaison Service- department of Orthopaedics and Traumasurgery, Delft, Netherlands*
[c]*Maastricht University Medical Centre, Department of Internal Medicine, Maastricht, Netherlands*
[d]*University Medical Center Utrecht, Department of Geriatrics, Utrecht, Netherlands*
[e]*Admiraal de Ruyter Ziekenhuis Goes, Department of Surgery, Goes, Netherlands*
[f]*Amsterdam UMC- location VUmc, Department of Rheumatology, Amsterdam, Netherlands*
[g]*Erasmus MC Rotterdam, Bone Center- department of Internal Medicine-section Endocrinology, Rotterdam, Netherlands*

Background/Introduction: During the initial phase of the COVID-19 pandemic there was no guidance of professional societies or guidelines on the organization of osteoporosis care in case of such a crisis and treatment relied on local ad-hoc strategies.

Purpose: Experiences from the current pandemic need to be taken into account for the near future and therefore a national multidisciplinary survey was carried out in the Netherlands.

Methods: A survey of 17 questions concerning the continuation of bone mineral density measurements by Dual Energy X-ray absorptiometry (DEXA), outpatient clinic visits and prescription of medication was sent to physicians, nurses, nurse practitioners and physician assistants working in the field of osteoporosis.

Results: 77 respondents finished the questionnaire, of whom 39 (50.6%) reported a decline in DEXA-scanning and 36 (46.8%) no scanning at all during the pandemic. There was an increase in remote consultations for both new and control patient visits (n=48, 62.3%; n=62, 81.7% respectively). Lower quality of care regarding fracture prevention was reported by more than half of the respondents (n=44, 57.1%). Treatment with intravenous bisphosphonates and denosumab was delayed according to 35 (45.4%) and 6 (6.3%) of the respondents, respectively.

Conclusion(s): During the COVID-19 pandemic, osteoporosis care almost completely arrested, especially because of the discontinuation of DEXA-scanning and closing of outpatient clinics. More than half of the respondents reported a substantial lower quality of osteoporosis care during the COVID pandemic. To prevent an increase in fracture rates and decreased patient motivation, adherence and satisfaction, standardization of osteoporosis care delivery in situations of crisis are needed.

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P123

Establishment of diagnostic model based on RF signal of radial quantitative ultrasound and analysis of its application value on osteoporosis diagnosis

Yue Ding[a], Baiyin Lei[b], Jiang Liu[c], Teng Ma[d], Qi Zhang[d], Yuan Fu[a], Wenqiang Luo[a]

[a]*SYSU, orthopedics, Guangzhou, China*
[b]*Shenzhen University, biomedical engineering, Shenzhen, China*
[c]*Southern University of Science and Technology, Computer Engineering and Science, Shenzhen, China*
[d]*Shenzhen Institutes of Advanced Technology, Biomedical and Health Engineering, Shenzhen, China*

Background/Introduction: Osteoporosis is a metabolic osteopathy syndrome characterized by decreased bone mass and destruction of bone microstructure, which can lead to increased bone brittleness and prone to fracture. The incidence of osteoporosis increases significantly with age. As people's average life span increases, there are more and more osteoporosis patients. At present, the standard diagnostic methods of osteoporosis are dual energy X-ray absorptiometry (DXA). However, DXA is expensive and has ionizing radiation to the human body, so it is not suitable as a screening method. Bone quantitative ultrasound (QUS) has the potential to be used as a screening method, but its diagnostic accuracy is not sufficient.

Purpose: This study explored the use of ultrasound radio frequency (RF) signals combined with artificial intelligence (AI) analysis to improve the diagnostic efficiency of QUS for osteoporosis.

Methods: The proposed method uses the RF signals as inputs in the AI analysis. The dataset consisted of 342 ultrasound RF signals, of which 99 were osteoporosis, 150 were osteopenia and 93 were normal as determined by DXA. The RF signals were divided randomly into training, and testing groups. Receiver operating characteristic curves (ROC) were drawn to compare the diagnostic efficiency of the RF signals and conventional ultrasound parameter (speed of sound, SOS).

Results: The sensitivity, specificity, and accuracy of the AI in diagnosis osteoporosis were 65.42%, 88.59% and 82.96%, respectively; for SOS, the corresponding values were 54.55%, 76.54% and 70.18%, respectively. The area under the receiver operating characteristic curve (AUC) was also higher for RF signals than SOS (AUC=0.89 vs. 0.67).

Conclusion(s): This preliminary study initially indicated that the proposed method of using ultrasound RF signals and AI was more accurate at diagnose osteoporosis than conventional ultrasound methods, and may offer a significant direction for osteoporosis screening.

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P124

Increasing the number of dexa-scans will improve health care outcome for patients with an increased fracture risk

Janneke Witteveen[a], Saskia Boonzajer Flaes[b], Pé Mullenders[b], Joop van den Bergh[c], Iris Groeneveld[b]

[a]*Medical Center Leeuwarden, Department of Internal Medicine, Leeuwarden, Netherlands*
[b]*National Health Care Institute, Department of Health Care, Diemen, Netherlands*
[c]*VieCuri Medical Centre, Department of Internal Medicine, Venlo, Netherlands*

Background/Introduction: One of the objectives of the Appropriate Care program ('Zinnige Zorg') of the National Health Care Institute of the Netherlands is to ensure appropriate care for patients with osteoporosis in the Netherlands, together with involved parties.

Purpose: The aim was to identify the number of patients 50 years and older with a recent fracture who underwent Dexa-scans and laboratory tests. We also identified the number of patients who received anti-osteoporosis treatment after a Dexa-scan.

Methods: To define appropriate care we used the current multidisciplinary Dutch osteoporosis guideline. To measure the care

provided, we used diagnosis-treatment-combination (DBC)-information-system (DIS), which contains declaration data of care activities for all Dutch citizens. These declaration data are provided by the Dutch Healthcare Authority. We used the data of the Medicine and Aids Information Project (GIP database), which contains extramural medication prescriptions of all Dutch citizens, to identify patients who started anti-osteoporosis medication. The GIP data are provided by the health insurance companies.

Results: Overall, in 2016 only 26% of 120.509 patients 50 years and older with a recent fracture, excluding head and face fractures, underwent a Dexa-scan in the year before up till the year after the fracture. 32% of the patient who underwent a Dexa-scan also had laboratory testing, according to the advice in the current Dutch guideline. 37% of the patients who underwent a Dexa-scan received anti-osteoporosis treatment. There was considerable practice variation between hospitals in the percentage of patients who underwent a Dexa-scan.

Conclusion(s): The number of Dexa-scans performed in patients 50 years and older with a recent fracture is too low. Once patients underwent a Dexa-scan they received laboratory testing and treatment according to current advice in the Dutch osteoporosis guideline. The main challenge to ensure appropriate care for patients with osteoporosis, or increased fracture risk, remains the capture of patients with a recent fracture.

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P125

Vertebral fractures in Ireland: A sub-analysis of the DXA HIP Project

John Carey[a], Lan Yang[b], Ee Erjiang[c], Wang Tingyan[d], Kelly Gorham[e], Rebecca Egan[e], Attracta Brennan[f], Mary Dempsey[g], Catherine Armstrong[e], Fiona Heaney[e], Ming Yu[h]
[a]*NUIG Galway, School of Medicine, Galway, Ireland*
[b]*NUIG- Galway, Engineering and Computer Science, Galway, Ireland*
[c]*Tsinghua University, Industrial Engineering and Computer Science, Bejing, China*
[d]*Oxford, Engineering, Oxford-, London, United Kingdom*
[e]*Galway University Hospitals, Rheumatology, Galway, Ireland*
[f]*NUIG- Galway, Engineering and Information Technology, Galway, Ireland*
[g]*NUIG- Galway, Engineering and Informatics, Galway, Ireland*
[h]*Tsinghua University, Computer Science and Industrial Engineering, Galway, Ireland*

Background/Introduction: The vertebrae are the commonest site of osteoporotic fracture osteoporosis, are associated with the highest mortality, and a huge illness burden. Some have questioned the value of screening for spine fractures, but studies from Europe, Latin-America, the USA and China show they are common and their prevalence increases with age. Epidemiologic data are limited for the Republic of Ireland.

Purpose: To examine the prevalence of vertebral fractures in men and women.

To compare the results to data from other countries.

To identify significant associations with common osteoporosis risks and tools.

Methods: We used GE Lunar DXA VFA scans from a sample of subjects included in a convenience cohort during a 1 year period for the presence of vertebral fractures (VF) following ethical approval. Demographics, major risk factors, osteoporosis treatments, prior fracture data, DXA T-scores and FRAX scores were collected. De-identified data were analysed using R software, version 3.5.1.

Results: 1324 subjects were included with a mean age of 69 years, including 1058 women and 266 men. 820 subjects had prevalent fractures, 96 VF. Mean BMD T-scores were: spine: -1.4, femoral neck: -1.5, total hip:-1.2, while mean FRAX scores were 15.4 and 4.8. 286 patients had VF, 152 single, 61 2, and 61 3 or more. 286 subjects had VF on their scans, including 152 single, 61 2, and 61 3 or more. The prevalence of VF increased with age from 11,5% in those aged 40-49 years to >33% among those aged >=80 years. Mean BMD was significantly lower at each measured site for VF subjects, while mean FRAX scores were higher, for both men and women, p <0.05.

Conclusion(s): VF are common in Irish men and women at high risk for fracture or with osteoporosis, and under-appreciated. VF are significantly associated with advancing age, higher FRAX scores and low BMD.

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P128

BMD accuracy errors in phantomless calibration of CT scans – a simulation study

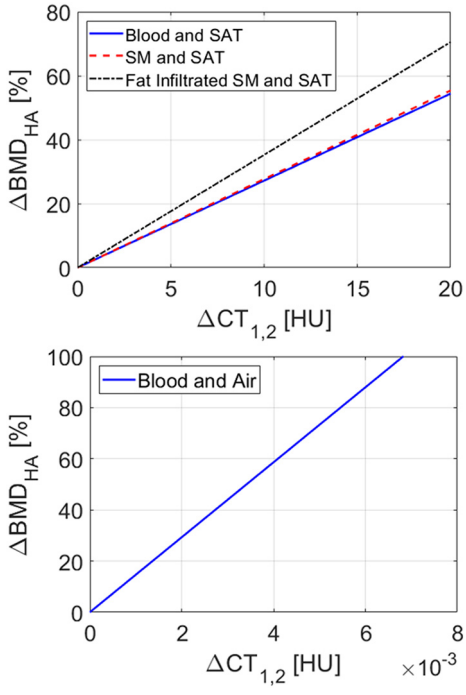
Stefan Bartenschlager, Klaus Engelke, Oliver Chaudry
Friedrich-Alexander University Erlangen-Nuremberg, Institute of Medical Physics, Erlangen, Germany

Background/Introduction: In internal calibration based opportunistic screening, two calibration materials (ICM) are required to convert CT values to BMD.

Purpose: In this study we simulated the impact of accuracy errors of the measured CT values on BMD.

Methods: Simulations used the base-material decomposition technique to obtain BMD of bone-equivalent hydroxyapatite (HA) from combinations of two ICMs, namely air/blood, subcutaneous adipose tissue (SAT)/blood, SAT/skeletal muscle (SM) and SAT/SM including 20% fat. Errors in measured CT values were assumed to originate from an inaccurate water calibration of the CT scanner or an impact of plagues on blood, edema on SAT and unknown fat content on SM. In the simulations, the accuracy BMD error in % was calculated as a function of the error of the measured CT values. For both ICMs identical CT errors were assumed.

Results: Lowest BMD errors were obtained for SAT/blood or SAT/SM (Figure). Increased fat content of SM increased BMD errors. Due to low density of air, BMD errors for air/blood were much higher. There was no impact of the water miscalibration.



Conclusion(s): Even for SAT/blood or SAT/SM an error of the CT measurement by just 5HU results in a BMD accuracy error of 14%. Air should not be used as ICM. The use of SM may also result in larger BMD errors due to unknown fat content. The important effect of internal calibration on longitudinal precision errors still needs to be evaluated but will largely depend on the longitudinal stability of the ICMs.

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P129

High-resolution three-dimensional microstructural analysis of cortex of the superolateral femoral neck in men reveals critical subregion

Ana Cirovic[a], Aleksandar Cirovic[a], Vladimir Zivkovic[b], Slobodan Nikolic[b], Petar Milovanovic[a], Marija Djuric[a], Danijela Djonic[a]
[a]*Faculty of Medicine- University of Belgrade, Institute of Anatomy, Belgrade, Serbia*
[b]*Faculty of Medicine- University of Belgrade, Institute of Forensic Medicine, Belgrade, Serbia*

Background/Introduction: Fractures of the femoral neck usually start in the superolateral neck. Superolateral neck has been always considered as uniform while epidemiological data suggest that prevalence of subcapital, transcervical and basicervical fractures is not uniform.

Purpose: Despite the fact that men face higher mortality risk than women after femoral neck fracture occurs, cortical microarchitectural variations of the superolateral femoral neck were insufficiently studied.

Methods: By using microcomputer tomography (10μm pixel size) we scanned the full length of each superolateral neck of forty-six proximal femora that were taken from healthy men during routine autopsies at the Institute of Forensic Medicine [51.3 SD: ± 20.4]. All proximal femurs were divided into three age- groups: young (21–39 years; N=14), middle-aged (40–60 years; N=12), and aged groups (61–86 years; N=20) Superolateral neck of each specimen was divided into the three equally long subregions (subcapital, transcervical and basicervical) and for each subregion we analyzed cortical thickness (Ct.Th) and cortical porosity (Ct. Po). Institutional Ethics Committee approved the research.

Results: Cortical thickness and cortical porosity varied significantly among the all three subregions ($p<0.001$). Basicervical subregion had thicker and less porous cortex compared to subcapital cortex ($p<0.001$), we also found that cortex of transcervical subregion is also thicker and less porous than cortex of the subcapital subregion ($p<0.001$). Transcervical and basicervical subregions did not differ in cortical thickness, ($p=0.092$) nevertheless cortex of transcervical subregion was significantly more porous than cortex of basicervical subregion ($p=0.024$). Cortical thickness declined whereas cortical porosity increased significantly with advanced age. ($p=0.035$, $p=0.006$) Aged individuals had thinner and more porous cortex compared to young group ($p=0.037$, $p=0.007$).

Conclusion(s): Microarchitecture of cortical bone of the superolateral neck varied among subregions. Subcapital subregion is least thick and highly porous compared to other two subregions while basicervical region has most favorable microarchitectural properties.

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P130

Ex vivo analysis of cortical microarchitecture of the distal clavicle: Implications for surgical management of fractures

Aleksandar Cirovic[a], Ana Cirovic[a], Marko Vujacic[b], Danijela Djonic[a], Marija Djuric[a], Petar Milovanovic[a]
[a]*Faculty of Medicine- University of Belgrade, Institute of Anatomy, Belgrade, Serbia*
[b]*Faculty of Medicine- University of Belgrade, Institute for Orthopaedic Surgery "Banjica", Belgrade, Serbia*

Background/Introduction: Prevalence of distal clavicle fractures substantially increases with advanced age, particularly in women. Considering atrophy of the surrounding muscles in the elderly, fracture resistance of distal clavicle depends on the strength of cortical bone. Cortical thickness and porosity are two main determinants of cortical bone strength.

Purpose: The purpose was to evaluate regional variations in cortical thickness and porosity of the distal clavicle to determine the microarchitectualy preferential places for placing screws during surgery for distal clavicle fracture.

Methods: Distal ends of eleven clavicles (6 men, 5 women; age: 81.9 ± 15.1 years) were scanned by microcomputed tomography at 10 μm resolution. First, we analyzed cortical thickness and porosity of each 500-μm area across the superior surface of distal clavicle at the level of conoid tubercle. Subsequently, we divided the full width of distal clavicle to three subregions with equal number of 500-μm areas (anterior, middle, and posterior) and analyzed cortical porosity and cortical thickness. Institutional Ethics Committee approved the research.

Results: We found the largest number of low-thickness and high-porosity areas in the anterior conoid subregion. Cortical porosity and cortical thickness varied significantly among the three subregions ($p<0.001$). Cortex of the anterior conoid subregion was more porous and thinner compared with the cortex of the middle ($p<0.001$, $p=0.002$, respectively) and posterior conoid subregions ($p<0.001$, $p=0.004$, respectively), particularly in women ($p<0.001$, $p=0.01$, respectively). Independent of the subregion, women had a more porous cortex ($p=0.03$) but similar thickness ($p=0.227$) compared with men.

Conclusion(s): Due to high cortical porosity and low thickness, the anterior conoid subregion should be avoided during screw placement, if possible, particularly in women. Our findings are also in accordance with epidemiological data about the predominance of distal clavicle fractures in women.

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P131

High-resolution three-dimensional microstructural analyzes of cortex of the superolateral femoral neck in men reveals critical subregion

Ana Cirovic[a], Aleksandar Cirovic[a], Vladimir Zivkovic[b], Slobodan Nikolic[b], Petar Milovanovic[a], Marija Djuric[a], Danijela Djonic[a]
[a]*Faculty of Medicine- University of Belgrade, Institute of Anatomy, Belgrade, Serbia*
[b]*Faculty of Medicine- University of Belgrade, Institute of Forensic Medicine, Belgrade, Serbia*

Background/Introduction: Fractures of the femoral neck usually start in the superolateral neck. Superolateral neck has been always considered as uniform while epidemiological data suggest that prevalence of subcapital, transcervical and basicervical fractures is not uniform.

Purpose: Despite the fact that men face higher mortality risk than women after femoral neck fracture occurs, cortical microarchitectural variations of the superolateral femoral neck were insufficiently studied.

Methods: By using microcomputer tomography (10μm pixel size) we scanned the full length of each superolateral neck of forty-six proximal femora that were taken from healthy men during routine autopsies at the Institute of Forensic Medicine [51.3 SD: ± 20.4]. All proximal femurs were divided into three age- groups: young (21–39 years; N=14), middle-aged (40–60 years; N=12), and aged groups (61–86 years; N=20) Superolateral neck of each specimen was divided into the three equally long subregions (subcapital, transcervical and basicervical) and for each subregion we analyzed cortical thickness (Ct.Th) and cortical porosity (Ct. Po). Institutional Ethics Committee approved the research.

Results: Cortical thickness and cortical porosity varied significantly among the all three subregions ($p<0.001$). Basicervical subregion had

thicker and less porous cortex compared to subcapital cortex ($p<0.001$), we also found that cortex of transcervical subregion is also thicker and less porous than cortex of the subcapital subregion ($p<0.001$). Transcervical and basicervical subregions did not differ in cortical thickness, ($p=0.092$) nevertheless cortex of transcervical subregion was significantly more porous than cortex of basicervical subregion ($p=0.024$). Cortical thickness declined whereas cortical porosity increased significantly with advanced age. ($p=0.035$, $p=0.006$) Aged individuals had thinner and more porous cortex compared to young group ($p=0.037$, $p=0.007$).

Conclusion(s): Microarchitecture of the cortical bone of the superolateral neck varied among subregions. Subcapital subregion is least thick and highly porous compared to other two subregions while basicervical region has most favorable microarchitectural properties.

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P132

Pronounced microarchitectural trabecular variations within the inferomedial femoral neck may potentially contribute to postoperative complication occurrence: A cadaveric study

Ana Cirovic[a], Aleksandar Cirovic[a], Vladimir Zivkovic[b], Slobodan Nikolic[b], Petar Milovanovic[a], Marija Djuric[a], Danijela Djonic[a]
[a]*Faculty of Medicine- University of Belgrade, Institute of Anatomy, Belgrade, Serbia*
[b]*Faculty of Medicine- University of Belgrade, Institute of Forensic Medicine, Belgrade, Serbia*

Background/Introduction: One of the parameters that may determine the direction of the fracture line propagation is the property of the trabecular bone. In majority of the studies have been showed that inferomedial neck is the place where fracture line terminates. If the fracture line terminates in the inferomedial neck close to greater trochanter (basicervical subregion of inferomedial neck) than shear forces are present and the angle of the fracture is high.

Purpose: Better understanding of the trabecular variations within the inferomedial neck subregions may be helpful to understand the fracture line propagations, nevertheless trabecular variations within the inferomedial neck are poorly evaluated.

Methods: By using microcomputer tomography (scanning pixel size: 10μm) we scanned the full length of each inferomedial neck of forty-one proximal femora that were obtained from healthy men during routine autopsies at the Institute of Forensic Medicine [age range: 51 years ± 20]. Inferomedial neck of each specimen was divided into the three equally long subregions (subcapital, transcervical and basicervical) and for each subregion following parameters were determined: bone volume fraction (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), structure model index (SMI). Institutional Ethics Committee approved the research.

Results: Microstructural parameters varied significantly among three subregions of the inferomedial neck ($p<0.001$). Subcapital subregion of inferomedial neck had significantly higher BV/TV, Tb.Th, Tb.N and lower Tb.Sp, SMI compared to transcervical subregion ($p<0.001$) and basicervical subregion ($p<0.001$). Transcervical subregion had higher BV/TV, Tb.Th and Tb.N and lower Tb.Sp and SMI compared to basicervical sub-region ($p<0.001$).

Conclusion(s): Apart from the place of the fracture origin, if the fracture propagates towards the least resistant subregion of the inferomedial neck than the fracture line may end in basicervical subregion of the inferomedial neck due to its weak microarchitectural properties and consequently favors complication occurrence.

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P133

Mapping of cortical porosity and thickness along the femoral neck identifies candidate critical spots for hip fracture in older women

Aleksandar Cirovic[a], Ana Cirovic[a], Danica Cvetkovic[b], Vladimir Zivkovic[b], Slobodan Nikolic[b], Danijela Djonic[a], Marija Djuric[a], Petar Milovanovic[a]
[a]*Faculty of Medicine- University of Belgrade, Institute of Anatomy, Belgrade, Serbia*
[b]*Faculty of Medicine- University of Belgrade, Institute of Forensic Medicine, Belgrade, Serbia*

Background/Introduction: Although several studies showed inter-individual differences in femoral neck cortical microstructure, intra-individual variations in femoral cortical bone are unknown.

Purpose: Microarchitecturally weak cortical bone, which may easily fracture, may be masked if surrounded by low porous and thick cortex. So far no study evaluated superolateral femoral neck by analyzing successively small areas.

Methods: Here we mapped cortical porosity (Ct.Po) and thickness (Ct.Th) along the superolateral femoral neck to identify "critical" spots where a fracture could start. The entire superolateral femoral neck obtained from 14 women (age: 77.1±9.8 years) at autopsy was scanned by microcomputed tomography. We mapped Ct.Po and Ct.Th values per smaller segments (500-μm-thick) along the neck. Institutional Ethics Committee approved the research.

Results: Not only did Ct.Po and Ct.Th differ between individuals, but they also varied substantially among the analyzed segments along the individual's neck, showing multiple critical spots (segments with high porosity and/or low thickness). Even 88% and 48% of cortical segments exceeded 75th and 90th percentile of individual's Ct.Po, respectively, in at least one individual. Although critical spots tended to accumulate closer to the femoral head, they were also present at other examined subregions.

Conclusion(s): We revealed tremendous diversity of Ct.Po and Ct.Th among the segments of the superolateral femoral neck. While the highest ratio of critical spots was found in the subcapital subregion, we observed a number of critical spots in other subregions as well, which provides the anatomic basis for explaining the fracture initiation at various sites of the superolateral neck. Given that fracture likely starts at a critical spot rather than at the entire region, average Ct.Po and Ct.Th of the neck should be interpreted with caution because they may not be fully representative of the fracture risk.

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P137

Temporal trends and factors associated with vitamin D prescription and intake

Giovanni Adami[a], Davide Gatti[a], Ombretta Viapiana[a], Pietro Olivi[b], Francesco Bertoldo[c], Angelo Fassio[a], Maurizio Rossini[a]
[a]*University of Verona, Rheumatology Unit, Verona, Italy*
[b]*University of Verona, Orthopedic Unit, Verona, Italy*
[c]*University of Verona, Bone Metabolism and Osteoncology Unit, Verona, Italy*

Background/Introduction: Compliance to the treatment of patients with osteoporosis is scarce. Understanding the factors associated with low vitamin D intake and prescription might help design future interventions aimed to improve adherence.

Purpose: This study aims to investigate the temporal trends and factors associated with vitamin D prescription and intake.

Methods: Using a web-based fracture risk-assessment tool, we collected demographic, densitometric and clinical data of women at

high risk for fractures. To determine the factors associated with low vitamin D intake we ran a multivariable logistic regression analysis. We described the public general interest in the term "vitamin D" using the Google Trends tool.

Results: 12,419 women were included in the study. 6,748 (54.4%) individuals had a prevalent fragility fracture and 8,950 (72.1%) individuals were on <800 IU of vitamin D per day and 11,434 (92.1%) were taking <1,200 IU of vitamin D per day. The factors associated with vitamin D intake were evaluated with a multivariable logistic regression analysis, which results are presented in Table 1. We found that low BMD levels, the presence of associated comorbidities and glucocorticoid utilization were associated with greater vitamin D intake. Overall, the internet interest in vitamin D was higher during the winter season (Figure 1).

Table 1. Binary logistic regression to predict the risk of low vitamin D intake (<800 IU/day)

>800 IU day REF		aOR (95% CI)	p value
400-800 IU day	Age	1.001 (0.995-1.007)	NS
	T-score	**1.422 (1.342-1.508)**	<0.001
	No comorbidities	REF	
	Rheumatoid arthritis	**0.732 (0.583-0.920)**	<0.001
	Psoriatic arthritis	**0.362 (0.218-0.607)**	<0.001
	Connective tissue disease	**0.630 (0.486-0.816)**	<0.001
	No cigarette smoking	REF	
	Cigarette smoking	1.040 (0.893-1.212)	NS
	Alcohol <3 unit day	REF	
	Alcohol ≥3 unit day	1.201 (0.660-2.187)	NS
	No glucocorticoids	REF	
	Glucocorticoids 2.5-5 mg/day	**0.688 (0.550-0.860)**	<0.001
	Glucocorticoids ≥5 mg/day	**0.739 (0.554-0.986)**	<0.001
	No falls	REF	
	Falls	0.875 (0.754-1.015)	NS
250-400 IU day	Age	0.994 (0.985-1.002)	NS
	T-score	**1.223 (1.117-1.339)**	<0.001
	No comorbidities	REF	
	Rheumatoid arthritis	**0.670 (0.463-0.970)**	<0.001
	Psoriatic arthritis	**0.300 (0.119-0.752)**	<0.001
	Connective tissue disease	**0.651 (0.435-0.975)**	<0.001
	No cigarette smoking	REF	
	Cigarette smoking	**1.371 (1.104-1.702)**	<0.001
	Alcohol <3 unit day	REF	
	Alcohol ≥3 unit day	1.824 (0.865-3.887)	NS
	No glucocorticoids	REF	
	Glucocorticoids 2.5-5 mg/day	0.930 (0.666-1.297)	NS
	Glucocorticoids ≥5 mg/day	0.743 (0.464-1.189)	NS
	No falls	REF	
	Falls	0.934 (0.741-1.176)	NS
<250 IU day	Age	**0.993 (0.988-0.998)**	<0.01
	T-score	**1.139 (1.079-1.202)**	<0.001
	No comorbidities	REF	
	Rheumatoid arthritis	**0.645 (0.522-0.795)**	<0.001
	Psoriatic arthritis	**0.553 (0.376-0.812)**	<0.001
	Connective tissue disease	**0.697 (0.558-0.871)**	<0.001
	No cigarette smoking	REF	
	Cigarette smoking	**1.339 (1.171-1.532)**	<0.001
	Alcohol <3 unit day	REF	
	Alcohol ≥3 unit day	1.267 (0.749-2.145)	NS
	No glucocorticoids	REF	
	Glucocorticoids <5 mg/day	0.964 (0.796-1.168)	NS
	Glucocorticoids ≥5 mg/day	0.946 (0.736-1.215)	NS
	No falls	REF	
	Falls	**0.615 (0.530-0.712)**	<0.001

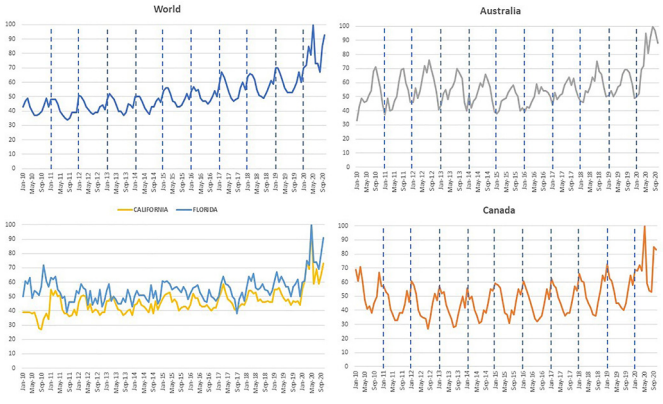

Figure 1. Google trends interest in the term "vitamin D" from January 2010 to October 2020. Interrupted lines in January every year.

Conclusion(s): Low vitamin D intake (<800 IU day) was common in our cohort of women at high risk for fractures. The factors associated with greater vitamin D intake were: having low BMD levels, associated rheumatic diseases and glucocorticoid use. Falls were associated with lower risk of receiving very low dose of vitamin D (<250 IU day). Smoking status was associated with lower vitamin D intake.

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P138

Risk factors for imminent fractures: A substudy of the Frisbee cohort

Laura Iconaru[a], Michel Moreau[b], Felicia Baleanu[c], Virginie Kinnard[d], Alexia Charles[d], Aude Mugisha[e], Murielle Surquin[e], Florence Benoit[e], Rafik Karmali[c], Marianne Paesmans[b], Jean-Jacques Body[c], Pierre Bergmann[f]
[a]*CHU Brugmann, Endocrinology, Brussels, Belgium*
[b]*Institute Bordet, Data Centre, Bruxelles, Belgium*
[c]*CHU Brugmann, Endocrinology, Bruxelles, Belgium*
[d]*CHU Brugmann, Laboratoire de Recherche Translationnelle, Bruxelles, Belgium*
[e]*CHU Brugmann, Internal Medicine, Bruxelles, Belgium*
[f]*CHU Brugmann, Nuclear Medicine, Bruxelles, Belgium*

Background/Introduction: The risk of a recurrent fragility fracture is maximal during the first two years following an incident fracture.

Purpose: In this prospective cohort study, we looked at the incidence of recurrent fractures within two years after a first incident fracture and we assessed independent clinical risk factors increasing this imminent fracture risk.

Methods: 3560 postmenopausal women recruited were surveyed yearly for the occurrence of fragility fractures. We identified patients who sustained a fracture during the first two years following an incident fracture. We quantified the risk of a new fracture and assessed independent CRFs, associated with an imminent fracture at various sites.

Results: Among the 3560 postmenopausal women, aged 60 to 85 years, we validated 814 first incident fragility fractures.Among patients with any type of index fracture, 8.5% [95% CI, 6.6-10.5%] had a subsequent fracture by 12 months and 15.4% [12.0-17.9%] by 24 months.

A recent fracture was a significant CRF for an imminent fracture (OR (95% CI): 3.7(2.4-5.7) [p<0.0001]). The incidence of an imminent fracture was higher in subjects above 80 years (p<0.001). Other CRFs highly predictive in a multivariate analysis were osteoporosis (p<0.01), a central fracture as the index fracture (p<0.01) and presence of comorbidities (p<0.05), with likelihood ratios of 1.9, 1.9 and 2.2, respectively. An imminent fracture was better predicted by a central fracture (p<0.01) than by a MOF. The HR was the highest for a central fracture (HR (95%CI) of 2.6 (1.3-5.3) [p<0.01] at one year and of 2.4 (1.4-4.0) [p<0.001] at two years).

Conclusion(s): A recent fracture, older age, osteoporosis, comorbidities and fracture site were associated with an imminent fracture risk. Central fractures were more predictive for an imminent fracture than MOFs. These findings could be a first step in the development of a model to predict an imminent fracture and select patients at need of immediate and appropriate treatment.

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P139

Lumbar spine bone mineral density and trabecular bone score-adjusted FRAX, but not FRAX without bone mineral density, identify subclinical carotid atherosclerosis

Jessica Pepe, Giada Della Grotta, Rachele Santori, Viviana De Martino, Marco Occhiuto, Mirella Cilli, Salvatore Minisola, Cristiana Cipriani
Sapienza Università di Roma, Department of Clinical- Internal-Anesthesiology and Cardiovascular Sciences, Rome, Italy

Background/Introduction: Osteoporosis and atherosclerosis share common risk factors. Preclinical atherosclerosis can be assessed by carotid intima media thickness (cIMT) measurement, a widely accepted marker of atherosclerosis and predictor of future vascular events.

Purpose: Aim of this study was to test if FRAX (which is an algorithm that can identify subjects at risk of fracture), without or with BMD values, also adjusted for trabecular bone score (TBS) was able to identify subclinical atherosclerosis, evaluated by measurement of cIMT ≥0.9 mm, as compared to DXA values.

Methods: Ninety postmenopausal women underwent DXA measurement and cIMT evaluation. For each patient, the FRAX algorithm for major osteoporotic fracture (M) and for hip fracture (H) without BMD was computed, together with FRAX with BMD and TBS-adjusted FRAX. Serum levels of osteoprotegerin, sRANKL, and interleukin-6 were also measured.

Results: There were no differences in anthropometric parameters and cardiovascular risk factors between subjects with cIMT ≥0.9 mm (35% of subjects, group A) compared to those with cIMT <0.9 mm (group B). The prevalence of osteoporosis and FRAX BMD, TBS-adjusted FRAX both for M and H were higher in group A compared to group B. The best ROC curves to identify subjects with a cIMT ≥0.9 mm were: lumbar spine T-score, with a threshold of -2.5 SD (area under the curve, AUC 0.64; $p = 0.02$) with a sensibility of 50% and a specificity of 76%; TBS-adjusted FRAX H with a sensibility of 50% and a specificity of 72% (AUC 0.64; $p = 0.01$ with a threshold of 3%). Interleukin-6 positively correlated with FRAX BMD H and M.

Conclusion(s): FRAX without BMD does not identify subclinical carotid atherosclerosis, while lumbar spine T-score and TBS-adjusted FRAX H similarly detected it with higher specificity for T-score. Our study highlights the interplay between bone and vascular system beyond common identifiable clinical risk factors.

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P141

Prevalence of vertebral fractures and non-fracture deformities in young healthy men

Stephanie De Smet, Hans-Georg Zmierczak, Stefan Goemaere
Ghent University Hospital, Endocrinology, Ghent, Belgium

Background/Introduction: Osteoporosis and vertebral fractures (VF) are common in older men but data on VF prevalence in young adult men is limited.

Purpose: The aim of this study was to describe the prevalence of VF and non-fracture deformities in healthy young Flemish men.

Methods: In the population-based SIBLOS-SIBEX cohort (men recruited from population registries of communities around Ghent, Belgium), vertebral fracture assessment (VFA) of vertebrae T4 to L5 was performed using a Hologic QDR-4500A DXA device for imaging. VFA images of 674 men, 32 to 61 years old (mean age 46.3 years) were evaluated. Every image was read by 2 out of 3 participating investigators, blinded for the results of each other and for medical patient data. In the case of disagreements, second readings were performed to reach consensus. For grading VF and deformities, the semi-quantitative system of Genant was used. For discriminating VF from non-fracture deformities we used the mABQ paradigm.

Results: Due to lacking visualisation or insufficient imaging quality, 1097 out of 9436 vertebrae could not be evaluated, resulting in 8339 assessed vertebrae. We found 52 fractured vertebrae (0.6%), of which 18 were classified grade 1, 29 grade 2 and 5 grade 3. 401 (4.8%) vertebrae showed non-fracture deformities, 314 were scored grade 1, 87 grade 2 and none grade 3. 27 participants (4%) had 1 or more VF, 15 had 1 and 12 had 2 or more VF. Of these, 4 had grade 1, 19 grade 2 and 4 grade 3 fractures.

248 men (36,8%) had non-fracture deformities, 149 had 1 and 99 had 2 or more deformities. Of these, 179 had grade 1, 69 grade 2 and none grade 3 deformities.

Conclusion(s): The prevalence of VF in our cohort of healthy young men was 4%, while non-fracture deformity prevalence was 36,8%. The Genant semiquantitative method overestimates VF prevalence.

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P143

The Appropriate Care program in the Netherlands: Improving osteoporosis care and preventing future fractures

Iris Groeneveld[a], Celine Leenen[a], Saskia Boonzajer Flaes[a], Janneke Witteveen[b], Pé Mullenders[a]
[a]*National Health Care Institute, Department of Health Care, Diemen, Netherlands*
[b]*Medical Center Leeuwarden, Department of Internal Medicine, Leeuwarden, Netherlands*

Background/Introduction: The Appropriate Care program ('Zinnige Zorg') of the National Health Care Institute aims at enhancing the quality of care in the Netherlands, in collaboration with relevant parties from the field.

Purpose: To make agreements on improvement of the care trajectory of patients aged 50+ with a recent fracture.

Methods: First, expert clinicians representing scientific organisations involved in osteoporosis care, as well as representatives of the patient organisation and insurance companies, were invited to participate in the Appropriate Care program. Second, along with these 15 parties, research questions were defined on topics considered as potential bottlenecks in the care trajectory. Third, by analysis of declaration data of health care activities, these research questions were adressed. Last, the results of these analyses were discussed among all parties and agreements for improvement were made and published online.

Results: Among others, the analyses showed that the percentage of Dutch fracture patients 50+ who underwent dexa-scanning in 2016 was only 26%. All parties underlined that these numbers should be improved. Possible actions for doing so were agreed upon. They included, i.e.: design and distribute a graphical representation of the care trajectory among health professionals, inform fracture patients about the importance of dexa-scanning, make adjustments to the hospital electronic system in order to facilitate the appropriate ordering of dexa-scanning, emphasize the importance of these actions to colleagues and hospital boards. The National Health Care Institute will facilitate implementation, e.g. by organising meetings in which lessons learned will be exchanged, by involving new relevant parties, and by sending newsletters to all involved. Also, it monitors improvement, by yearly analysis of declaration data of health care activities.

Conclusion(s): Sound data-analyses and discussions with relevant parties led to well-considered agreements on better detection and treatment of patients with osteoporosis, and thereby contribute to a decline in future fractures.

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P144

Multimorbidity is associated with fragility fractures in women in Portugal

Anabela Barcelos[a,b,c], Helena Canhão[a,b,c,d], Ana Rodrigues[a,b,c,d]
[a]*EpiDoC Unit- CEDOC., NOVA Medical School- Universidade Nova de Lisboa., Lisbon, Portugal*
[b]*Comprehensive Health Research Center., Universidade NOVA de Lisboa., Lisbon, Portugal*

[c]NOVA National School of Public Health., Public Health Research Centre-Universidade NOVA de Lisboa., Lisbon, Portugal
[d]NOVA Medical School., Universidade Nova de Lisboa., Lisbon, Portugal

Background/Introduction: Osteoporosis is a public health problem worldwide responsible for fragility fractures (FF). Multimorbidity is very common in elderly patients and there is insufficient knowledge in Portugal about the association of multimorbidity in patients with prevalent FF.

Purpose: Evaluate the association between sociodemographic, lifestyles and chronic non communicable chronic diseases with a prevalent FF in portuguese women > 50 years old.

Methods: Women aged 50 years and older from the EpiReumaPt study (2011–2013), a nationwide, population-based study, were evaluated. Self-reported data regarding sociodemographic, FF and chronic non communicable diseases was collected through a semi-structured questionnaire. FF was defined as any self-reported low-impact fracture that occurred after 40 years of age. Women with prevalent FF were compared with women without prevalent FF. Descriptive, chi-square and Odds Ratio were estimated. All statistical tests were performed using the SPSS 26, considering the significance level of 5%.

Results: A total of 3.662 women with 50 years and more were included and 646 women self-reported a FF. The chronic non communicable disease more frequently self-reported among FF women was rheumatic and musculoskeletal disease (62.9%) followed by hypertension (58.8%) and mental disease (30.0%). There was a significant association between the existence of FF and hypertension (OR= 1.36 (1.15-1.62); p<0.0001) and with diabetes mellitus (OR= 1.36 (1.10-1.67); p-0.004), adjusted for age and rheumatic disease. The same results were found for other chronic diseases, after adjusted for age and rheumatic disease. There was an association between the existence of FF and lower education, but without statistical significance when adjusted for age and rheumatic disease. No association was found between prevalent FF and lifestyles.

Conclusion(s): Prevalent FF are associated with multimorbidity in women > 50 years of age. This should raise awareness to the need to have hospitals and community units prepared to implement integrated care units among fragility fracture patients.

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P145

The assessment of fracture risk and osteoporosis rate among patients over 50 years old undergoing medical rehabilitation

Larisa Marchenkova
National Medical Research Center of Rehabilitation and Balneology-Rehabilitation department for somatic patients, Rehabilitation department for somatic patients, Moscow, Russia C.I.S.

Background/Introduction: Taking a course of physical rehabilitation creates the prerequisites for falls and injuries in patients at high risk of fractures. Data on fracture risk and prevalence of osteoporosis in older patients starting medical rehabilitation can change the approach of doctors to the development of rehabilitation programs and the management of such patients.

Purpose: To assess the prevalence of osteoporosis, individual risk factors for osteoporosis as well as the proportion of people with high risk of osteoporotic low-energy fractures among patients over 50 years old undergoing treatment according to the "medical rehabilitation" profile.

Methods: The study group comprised of 600 patients (426 women and 174 men) aged 50 to 84 years, average age 64.25 ± 10.17 years, undergoing treatment in a rehabilitation department. This was a cross-sectional study in the form of unified questionnaire, including data concerning age, weight, height, BMI, clinical and rehabilitation diagnosis, anamnesis of the main disease, anamnesis vitae, presence of osteoporosis diagnosis in the anamnesis, its treatment, osteoporosis risk factors estimation. An assessment of 10-year probability of osteoporotic fractures was carried out using Russian model of online FRAX® calculator.

Results: 41.8% patients in the study sample had osteoporosis risk factors, including 31.2% of subjects had 3 risk factors or more. 38.0% patients showed a high fracture risk according to the FRAX calculator. 34.1% had a diagnosis of osteoporosis, and 45.8% already had osteoporotic fractures. Among those who did not undergo densitometry examination, 69.9% had a history of low-traumatic fractures, and only 58.5% of patients with an established diagnosis of osteoporosis and 26.8% of those at high risk of fractures received effective therapy for osteoporosis.

Conclusion(s): Population of patients over 50 years old undergoing rehabilitation is characterized by high frequency of osteoporosis and probability of fractures, and insufficient quality of osteoporosis verification and anti-osteoporotic therapy administration at the same time.

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P149

Independent external validation of FRAX® and Garvan fracture risk calculators: A sub-study of the FRISBEE cohort

Felicia Baleanu[a], Alexia Charles[b], Laura Iconaru[a], Virginie Kinnard[b], Jean-François Fils[c], Michel Moreau[d], Rafik Karmali[a], Murielle Surquin[e], Florence Benoit[e], Aude Mugisha[e], Marianne Paesmans[d], Michaël R. Laurent[f], Pierre Bergmann[g], Jean-Jacques Body[a]
[a]CHU Brugmann- Université Libre de Bruxelles, Endocrinology, Brussels, Belgium
[b]CHU Brugmann- Université Libre de Bruxelles, Clinical Research, Brussels, Belgium
[c]Ars Statistica, Ars Statistica, Brussels, Belgium
[d]Inst. J. Bordet- Université Libre de Bruxelles, Data Centre, Brussels, Belgium
[e]CHU Brugmann- Université Libre de Bruxelles, Department of Geriatrics, Brussels, Belgium
[f]University Hospitals Leuven, Centre for Metabolic Bone Diseases, Leuven, Belgium
[g]CHU Brugmann- Université Libre de Bruxelles, Department of Nuclear Medicine, Brussels, Belgium

Background/Introduction: Probabilistic models including clinical risk factors with or without bone mineral density (BMD) have been developed to estimate the 5- or 10-years absolute fracture risk.

Purpose: We investigated the performance of the FRAX® (Belgium) and Garvan tools in a well-characterized population-based cohort of 3560 postmenopausal, volunteer women, aged 60-85 years at baseline, included in the Fracture Risk Brussels Epidemiological Enquiry (FRISBEE) cohort, during 5 years of follow-up.

Methods: Baseline data were used to calculate the estimated 10-year risk of hip and major osteoporotic fractures (MOFs) for each participant using FRAX® (Belgium). We computed the five-year risk according to the Garvan model with BMD.

For calibration, the predicted risk of fracture was compared with fracture incidence across a large range of estimated fracture risks. The accuracy of the calculators to predict fractures was assessed using the area under the receiver operating characteristic curves (AUC).

Results: The FRAX® tool was well calibrated for hip fractures (slope 1.09, p<0.001; intercept -0.001, p=0.46) but it consistently underestimated the incidence of major osteoporotic fractures (MOFs) (slope 2.12, p<0.001; intercept -0.02, p=0.06). The Garvan tool was well calibrated for 'any Garvan' fractures (slope 1.05, p<0.001; intercept 0.01, p=0.37) but largely overestimated the observed hip fracture rate (slope 0.32, p<0.001; intercept 0.006, p=0.05).

The predictive value for hip fractures was better for FRAX® (AUC: 0.841, 95% CI 0.795-0.887) than for Garvan (AUC: 0.769, 95% CI 0.702-

0.836, p=0.01). The Garvan AUC for 'any Garvan' fractures was 0.721 (95% CI 0.693-0.749) and FRAX AUC for MOFs was 0.708 (95% CI 0.675-0.741).

Conclusion(s): In conclusion, in our cohort, FRAX® estimated quite well hip fractures but underestimated MOFs, while Garvan overestimated hip fracture risk, but showed a good estimation of 'any Garvan' fractures. Both models had a good discriminatory value for hip fractures but only a moderate discriminatory ability for MOFs or 'any Garvan' fractures.

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P150

The use of bisphosphonates to treat osteoporosis in patients with Lysinuric Protein Intolerance

Jos Draaisma, Anne Dittrich, Maaike de Vries
Radboudumc Amalia Children's hospital, Pediatrics, Nijmegen, Netherlands

Background/Introduction: 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. There are no guidelines for the prevention and therapy of osteoporosis in these patients.

Purpose: To propose the use of bisphosphonates for osteoporosis in patients with LPI.

Methods: Clinical description of a patient and review of literature.

Results: This 8-year old girl was born to non-consanguineous parents. She had a uneventful clinical 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.

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. An increased level of hydroxyproline in serum and urine was shown, suggesting an increased activity of osteoclasts, and decreased collagen synthesis in children and adolescents. 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.

Conclusion(s): Bisphosphonates can be used to treat osteoporosis in patients with LPI.

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P152

Increasing the use of anti-osteoporosis medication in patients who use glucocortcoids remains a challenge

Janneke Witteveen[a], Pé Mullenders[b], Saskia Boonzajer Flaes[b], Iris Groeneveld[b], Willem Lems[c]
[a]*Medical Center Leeuwarden, Internal Medicine, Leeuwarden, Netherlands*
[b]*National Health Care Institute, Health care, Diemen, Netherlands*
[c]*Amsterdam University Medical Center, Department of Rheumatology and Immunology, Amsterdam, Netherlands*

Background/Introduction: One of the objectives of the Appropriate Care program ('Zinnige Zorg') of the National Health Care Institute of the Netherlands is to ensure appropriate care for patients with osteoporosis in the Netherlands, together with the involved parties.

Purpose: The aim was to identify the number of patients who receive anti-osteoporosis medication next to glucocorticoids.

Methods: To define appropriate care we used the current multidisciplinary Dutch osteoporosis guideline. We used the data of the Medicine and Aids Information project (GIP database), which contains extramural medication prescriptions of all Dutch citizens, to identify patients who have used glucocorticoids, prednisolone equivalent of >7,5 mg per day, continuously for more than 3 months. The GIP data are provided by the health insurance companies. Using the GIP database we also identified patients who used anti-osteoporosis medication.

Results: In 2016 26.265 patients received a first prescription for glucocorticoids, prednisolone equivalent of >7,5 mg per day, of which 15.175 patients for prednisolone equivalent of >15 mg per day. Within 3 months 7.038 (27%) of the 26.265 patients who use >7,5 mg per day prednisolone equivalent and 5.162 (34%) of the 15.175 patients who use >15 mg per day prednisolone equivalent also use anti-osteoporosis medication. Within 2 years 9.265 (35%) of the 26.265 patients who use >7,5 mg per day prednisolone equivalent and 6.775 (45%) of the 15.175 patients who use >15 mg per day prednisolone equivalent also use anti-osteoporosis medication.

Conclusion(s): The number of patients who receive a prescription for anti-osteoporosis medication next to glucocorticoids is low in the Netherlands, despite the development of the 2011 guideline and the medical pharmaceutical decision rules for glucocorticoids used by pharmacists. Together with prescribers and pharmacist we need to explore new ways to increase the number of patients that receive anti-osteoporosis medication next to glucocorticoids.

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P153

The effect of food supplement with calcium and vitamin D3 on calcium homeostasis and falls incidence in patients with high fracture risk undergoing medical rehabilitation

Larisa Marchenkova, Vasileva Valeria
National Medical Research Center of Rehabilitation and Balneology-Rehabilitation department for somatic patients, Rehabilitation department for somatic patients, Moscow, Russia C.I.S.

Background/Introduction: Fall preventing and nutritional status improving are important for patients with high fracture risk undergoing physical rehabilitation.

Purpose: To evaluate the effect of long-term calcium and vitamin D3 intake on calcium homeostasis and fall's rate in patients with high fracture risk starting rehabilitation course.

Methods: The study enrolled 119 men and women aged 50-80 y.o. with high absolute fracture probability by FRAX who started medical rehabilitation. 41 patients have been receiving antiresorptive therapy already comprised group 1, other patients were randomized into groups 2 (n=39) and 3 (control, n=39). In groups 1 and 2, a food supplement containing calcium citrate 1000 mg and vitamin D3 600 IU was prescribed for 12 months. All patients undergo laboratory examination, food calcium intake and fall assessment at baseline, in 6 and 12 months.

Results: Daily calcium intake in the study sample (n=119) was 782.9±243.4 mg. Vitamin D deficiency was detected in 38.4% of the examined. An increase in 25(OH)D level was noted in groups 1 and 2 after 6 and 12 months ($p<0.01$). Patients in group 1 showed an increase in serum osteocalcin and calcium levels after 6 and 12 months ($p<0.05$). In group 3, there was an increase of PTH level after 6 ($p<0.05$) and 12 months ($p<0.01$), CTx and alkaline phosphatase after 12 months ($p<0.05$). In group 1, there was a decrease in proportion off fallen at least once patients after 6 months ($p = 0.026$) and in the total falls cases after 12 months ($p = 0.027$). Group 2 showed a decrease in fallen patients number after 6 and 12 months ($p=0.0034$) and in total falls number after 6 months ($p=0.0142$).

Conclusion(s): Prescription of dietary supplements containing calcium and vitamin D3 should be recommended as a part of complex rehabilitation of patients with high fracture risk.

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P155

Effect of flavanols on bone turnover markers in individuals with type 2 diabetes – a 3-month randomized placebo-controlled FLAVA trial

Komal Waqas[a], Mardin Rashid[b], Bram Eerden[b], Kirsten Berk[c], M. Carola Zillikens[a]
[a]*Erasmus University Medical Center, Bone Centre- Department of Endocrinology and Internal Medicine, Rotterdam, Netherlands*
[b]*Erasmus University Medical Center, Department of Internal Medicine, Rotterdam, Netherlands*
[c]*Erasmus University Medical Center, Department of Dietetics, Rotterdam, Netherlands*

Background/Introduction: Subjects with type 2 diabetes mellitus (T2DM) have increased fracture risk with higher bone mineral density, possibly related to increased advanced glycation end-products (AGEs) accumulation. Studies showed reduced AGEs formation and improved bone health when treated with flavanols but not in subjects with T2DM.

Purpose: To study the effect of flavanols supplementation on bone turnover markers (BTMs) in participants with T2DM.

Methods: 83 individuals with T2DM, aged 40-85years, with microalbuminuria were enrolled from 4 trial centers in Rotterdam into a randomized, double-blind, placebo-controlled trial with renal vascular health as primary outcome. Participants were randomized (1:1) to receive 200mg of monomeric and oligomeric flavanols (MOFs) or placebo for 3 months. Serum alkaline phosphatase (ALP), type I collagen crosslinked beta C-telopeptide (β-CTx) and type I procollagen-N-propeptide (P1NP) were measured at baseline and 3 months. ANCOVA was performed on rank transformed BTMs at 3months as outcome adjusting for baseline BTMs, group, age, sex and BMI.

Results: Baseline characteristics did not differ between the two arms. The adjusted mean change in BTMs at 3months was not different between placebo vs. MOFs arm: AP -0.059 (-0.262 – 0.145) vs. 0.060 (-0.135 – 0.356), p=0.41; β-CTx 0.013 (-0.205 – 0.231) vs. 0.100 (-0.109 – 0.310), p=0.53 and P1NP 0.091 (-0.080 – 0.262) vs. 0.030 (-0.134 – 0.195), p=0.61. Within group difference in the MOFs arm from baseline to 3 months did not show a significant change: ALP 80(56-140) to 80(47-152) U/L, β-CTx 0.18 (0.08-0.58) to 0.19 (0.07-0.71) μg/ml and P1NP 36 (16-110) to 38 (16-112) μg/ml. We also observed no within-group changes in the placebo arm.

Conclusion(s): Supplementation with 200mg flavanols during three months in individuals with T2DM did not result in changes in BTMs. Bone AGEs may not change in 3months, future studies are needed to show whether long term supplementation may positively affect BTMs in individuals with T2DM.

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P156

Successive anti-osteoporosis treatment after denosumab in the years 2011 till 2017

Janneke Witteveen[a], Saskia Boonzajer Flaes[b], Pé Mullenders[b], Harry van den Broek[c], Willem Lems[d], Iris Groeneveld[b]
[a]*Medical Center Leeuwarden, Department of Internal Medicine, Leeuwarden, Netherlands*
[b]*National Health Care Institute, Department of Health Care, Diemen, Netherlands*
[c]*Osteoporosis Patient Association, Board, Den Haag, Netherlands*
[d]*Amsterdam University Medical Center, Department of Rheumatology and Immunology, Amsterdam, Netherlands*

Background/Introduction: One of the objectives of the Appropriate Care program ('Zinnige Zorg') of the National Health Care Institute of the Netherlands is to ensure appropriate care for patients with osteoporosis in the Netherlands, together with the involved parties.

Purpose: The aim was to identify the number of patients who discontinued denosumab without successive anti-osteoporosis medication, and were therefore at risk of a rebound effect.

Methods: We used the data of the Medicine and Aids Information Project (GIP database), which contains extramural medication prescriptions of all Dutch citizens, to identify patients who use anti-osteoporosis medication. The GIP data are provided by the health insurance companies. We defined successive therapy as a prescription for another anti-osteoporosis medication within 9 months of the last denosumab prescription. For the analysis of successive therapy we have excluded patients who died within 2 years of stopping denosumab.

Results: Between 2011 and 2017, the number of patients who started with denosumab for the treatment of osteoporosis tripled from 1.598 to 4.600. Between 2011 and 2016, the number of patients that stopped denosumab without successive anti-osteoporosis medication steeply increased from 158 to 1.692. In 2016, 83% of the 2.043 patients that stopped using denosumab did not receive successive anti-osteoporosis medication. Of the 351 (17%) patients who did, 85% received oral bisphosphonates, 11% received teriparatide and 4% received zoledronic acid.

Conclusion(s): The number of patients who stopped denosumab without successive treatment steeply increased between 2011 and 2016. Therefore, a growing number of patients became at risk of a rebound effect. In 2019 the Royal Dutch Pharmacists Association together with the Dutch Association for Endocrinology and the Osteoporosis Patient Association have written a warning letter to prescribers and users of denosumab. We shall evaluate the effect during the coming years.

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P157

Longterm outcomes of rheumatoid arthritis patients with severe osteoporosis treated with either Teriparatide or antiresorptive treatment – an observational study

Barbara Hauser[a,b], Kathryn Berg[b], Justine Lambert[b], Stuart H. Ralston[a,b]
[a]*Nhs Lothian, Rheumatology, Edinburgh, United Kingdom*
[b]*Rheumatology and Bone Disease Unit- Centre for Genomic and Experimental Medicine- MRC institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, United Kingdom*

Background/Introduction: Patients with Rheumatoid Arthritis (RA) are at increased risk of fragility fractures compared with the general population.

Purpose: The aim of this study was to compare the efficacy of Teriparatide (TPTD) with anti-resorptive treatment (ART) in RA patients with severe spinal Osteoporosis (OP).

Methods: Observational study of postmenopausal women with RA and severe OP. Patients with a history of two vertebral fractures or a spinal BMD Tscore < -4 were offered either TPTD or either oral or parenteral ART. DEXA re-evaluation was usually performed after 2

and 5 years. Fracture incidence was assessed through retrospective case record review.

Results: We studied in total 59 RA patients, 29 patients received TPTD and 30 patients ART. Approximately half of TPTD patients (55.2%) had previously received bisphosphonates and 10.3% received low dose Prednisolone (mean dose=5.5±3.3 mg).

We found that TPTD treatment was superior to ART at increasing spine BMD after 2 years (+17.59±9.63% vs +3.19±4.99%, p value<0.001). Assessment after 5 years showed that spine BMD remained higher in the TPTD+ART group than in the ART group alone (18.0±11.6% vs 6.36±8.95%; p=0.019). There was no significant difference between the groups on hip BMD change at 2 or 5 years.

In the follow up period 7 (24.1%) TPTD patients sustained a new vertebral fracture versus 13 (43.3%) patients who received ART only (p=0.119). In total 28 (47.5%) patients sustained a new clinical fracture after the commencement of osteoporosis medication. The average time from treatment start to first fracture was 62.7±30.4 months.

Conclusion(s): This real-world study confirms that TPTD treatment in RA patients led to a significant greater increase in spinal BMD than antiresorptive medication alone which is in keeping with a trend of a lower vertebral fracture incidence in the TPTD group. Despite treatment however, almost half of RA patients sustained at least one further fracture after approximately 5 years.

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P158

Factors associated with postoperative acute kidney injury after surgery of osteoporotic hip fractures

Joon-Soon Kang[a], Young-Sook Park[b]
[a]*Inha University Hospital, Orthopaedic, Incheon, Korea- South*
[b]*Eulji University Hospital- Seoul Korea, Internal medicine, Seoul, Korea-South*

Background/Introduction: The worldwide population is steadily aging, thus causing an increase in the incidence of osteoporotic hip fractures. A previous report found that the main cause of death after hip fractures was cardiovascular-related. According to Partridge et al. acute kidney injury (AKI) after hip fractures had complications associated with mortality of the second most commonly.

Purpose: Acute kidney injury (AKI) is a rare but serious complication after hip fractures. The aim of this study was to evaluate the incidence and the risk factors of postoperative AKI after hip fractures. Methods:

Methods: From January 2011 to December 2016, 550 patients who underwent surgery of hip fractures at our institution were retrospectively reviewed. The incidence, mortality, and risk factors of postoperative AKI were investigated. Receiver operating characteristic curve analysis was conducted to evaluate the ability of markers in predicting AKI.

Results: The incidence of postoperative AKI was 4.4% (25 cases). The mean onset of postoperative AKI was 8.0 + 5.3 days and recovered after 7.0 + 4.2 days after the occurrence of AKI. Of 25 patients with AKI, 6 patients (24.0%) died within 1 year after surgery. The independent risk factors for postoperative AKI are the estimated blood loss (EBL) (odds ratio (OR) 1.64; 95% confidence interval (CI) 1.33–2.58; $p < 0.01$) and postoperative level of albumin (OR 1.77; 95% CI 1.52–2.74; $p < 0.01$). The cutoff value of the serum albumin was <2.8 g/dL with a sensitivity of 88.0% and a specificity of 77.1%. The cutoff value of EBL was <766.5 mL with a sensitivity of 84.0% and a specificity of 66.3%.

Conclusion(s): Postoperative AKI after hip fractures had a low incidence (4.4%) but high mortality (24.0%). The important risk factors for postoperative AKI are blood loss and postoperative serum albumin levels. Therefore, surgeons should attempt to adequate transfusion and albumin management

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P160

Reversal of Doxorubicin-induced bone loss by antioxidant supplement

Sunil Poudel[a], Marisol Izquierdo[b], M. Leonor Cancela[c], Paulo J. Gavaia[c]
[a]*University of Algarve, Faculty of Medicine and Biomedical Sciences FMCB- PhD Program in Biomedical Sciences- Centre of Marine Sciences, Faro, Portugal*
[b]*University of Laspalmas de Gran Canaria, Grupo de Investigación en Acuicultura, Gran Canaria, Spain*
[c]*University of Algarve, Faculty of Medicine and Biomedical Sciences FMCB- Centre of Marine Sciences, Faro, Portugal*

Background/Introduction: Doxorubicin (Dox) is widely used as a chemotherapeutic drug for the treatment of various cancers. Recently, several studies have reported that administration of doxorubicin-induces bone loss and results on secondary osteoporosis. The mechanism behind the bone-loss mediated by doxorubicin is not clear, but oxidative stress has been suggested as a potential cause.

Purpose: Antioxidants that can counteract the toxic effect of doxorubicin on bone would be helpful for the prevention of secondary osteoporosis.

Methods: Therefore, we used two antioxidants, Resveratrol (Res) and MitoTempo (MT) to counteract the negative effect of doxorubicin on bone using *in-vitro* and *in-vivo* models.

Results: MC3T3 cells were exposed to Res, MT, Dox alone or in-combination, and osteoblast differentiation and mineralization were analyzed. Both were reduced by Dox treatment and were significantly reversed by co-treatment with antioxidants. THP-1 cells were differentiated to osteoclasts while treated with Res, MT and Dox. Results showed that TRAP-positive cells were significantly increased on Dox treatment and significantly decreased on antioxidant treatments. For *in-vivo* trials, zebrafish and seabream were fed with diets supplemented with either antioxidants or Doxorubicin or both in combination. The incidence, distribution of skeletal deformities, stages of bone mineralization and development were examined. Survival of zebrafish larvae was significantly decreased under Dox treatment but significantly reversed upon co-treatment with antioxidants. Dox also delayed larvae development and significantly decreased their total length, both for zebrafish and seabream, but the latter was significantly reversed by co-treatment with antioxidants. Dox also increased the incidence of bone deformities and affected areas, and decreased bone mineralization, effects that were significantly reversed with an antioxidant co-treatment.

Conclusion(s): In conclusion, our results showed that antioxidant supplements effectively prevent doxorubicin-induced incidence of bone anomalies and mineralization defects in our models, thus suggesting that a combined therapy of Doxorubicin and antioxidants may be beneficial for preventing Doxorubicin-induced secondary osteoporosis in humans.

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P162

Identification of bone anabolic compounds of marine origin using a zebrafish pipeline

Joana Rosa[a], Alessio Carletti[a,b], Tatiana Varela[a,b], Paulo J. Gavaia[a,b], M. Leonor Cancela[a,b,c,d], Vincent Laizé[a]

[a]University of Algarve, Centre of Marine Sciences CCMAR, Faro, Portugal
[b]University of Algarve, Faculty of Medicine and Biomedical Sciences FMBS, Faro, Portugal
[c]University of Algarve, Centre for Biomedical Research CBMR, Faro, Portugal
[d]University of Algarve, Algarve Biomedical Center ABC, Faro, Portugal

Background/Introduction: Low bone mass density associated disorders, such as osteoporosis, affect millions of people worldwide and result in a massive economic burden for society. Current therapeutics are limited and have issues related to costs, efficacy and long-term use. Although still largely unexplored, marine biodiversity represents a promising source of natural bioactives, in particular compounds with the capacity to improve skeletal status that can be used for the development of the next generation of therapies. It is therefore of utmost interest to screen marine resources for osteoactive compounds.

Purpose: Using several *in vivo* zebrafish screening systems, as an alternative to lower troughput mammalian systems, this study aimed to identify marine osteogenic extracts and evaluate their effect on bone growth and mineralization.

Methods: Extracts and fractions derived from a variety of marine organisms - cyanobacteria, actinobacteria, planctomycetes, microalgae, seaweeds and halophytes - were prepared using different solvents and assessed for their capacity to increase the growth of the opercular bone[1], *de novo* bone formation[2] and the extracellular matrix mineralization[3].

Results: From a total of 160 fractions evaluated, 24 were shown to increase up to 60% the opercular area of zebrafish larvae and 10 were selected and further tested for their effect on bone regeneration. From those, 6 remarkably increased the mineralized area of newly formed rays, but also affected thickness and patterning. Fractions were additionally tested *in vitro* and several of them were found to stimulate (up to 3.5 folds) extracellular matrix mineralization.

Conclusion(s): Our data confirms the potential of marine organisms as a source of osteogenic and mineralogenic compounds, but also the suitability of the zebrafish as a first approach for bioactive screening. Promising extracts are being fractionated towards the identification of osteoactive compounds.

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P165

Osteoporosis and recent-onset diabetes after liver transplantation: A common link?

Soledad Librizzi[a], Gonzalo Allo Miguel[a], Gema Villa[a], Mercedes Aramendi[b], Federico Hawkins[c], Miguel León Sanz[a], Guillermo Martínez Díaz-Guerra[a]
[a]12 de Octubre University Hospital, Endocrinology, Madrid, Spain
[b]12 de Octubre University Hospital, Laboratory, Madrid, Spain
[c]12 de Octubre University Hospital, Research Institute, Madrid, Spain

Background/Introduction: Osteoporosis and diabetes mellitus are two major chronic disorders which prevalence increases after liver transplantation (LT). Pre-clinical evidence has established that an interplay exists between glucose and bone metabolism.

Purpose: Assess the prevalence of new onset diabetes (NOD) and osteoporosis in patients after liver transplantation and evaluate the relationship between bone mass/ microarchitecture and body composition parameters and insulin resistance index.

Methods: 111 patients (70 males,41 females) with LT without previous diagnosis of DM pre-transplantation. Bone mineral density (BMD) and body composition analysis were performed with DXA. Trabecular Bone Score (TBS) was obtained from lumbar spine scan with TBS iNsight2.0 software. Biochemical analysis: serum osteocalcin, beta-CTX and sclerostin. HOMA (insulinrresistance index), calculated from fasting glucose and serum insulin.

Results: Mean age 61.5±10.6years, mean BMI: 26.7±4.8Kg/m2. NOD criteria (HbA1c >6.5% or fasting glucose >126 mg/dl, or use of hypoglycemic drugs/insulin after transplantation) was present in 35% of the group. Also, 18% showed osteoporosis and 71% osteopenia. Degraded TBS (<1,200) was found in 19.8% of the patients. No significant differences in percentage of osteoporosis, osteopenia or degraded TBS were found between diabetic and non-diabetic subjects. Fat-free mass was significantly increased (p=0.019) and percentage of fat mass decreased (p=0.002) in diabetic subjects compared with non-diabetic. In diabetic subgroup, HOMA index was significantly increased (4.19±2.97 vs 2.89±2.18,p=0.01) and serum beta-CTX significantly decreased compared with non-diabetic (0.36 ±0.20ng/ml vs 0.50±0.28ng/ml,p=0.003). In multiple linear regression analysis, fat-free mass and serum sclerostin were positively associated with lumbar spine BMD (b-coefficients 0,41;p=0,004 and 0,37;p=0,006), whereas serum osteocalcin was inversely correlated (b-coefficient -0,31; p=0,024).

Conclusion(s): In liver transplantation patients, osteoporosis and NOD are prevalent diseases. As it happens in Type 2 diabetes, NOD in transplanted patients is associated with insulin resistance and low bone turnover. However, bone microarchitecture seems to be relatively unaffected. Relationship between BMD, fat-free mass and serum sclerostin deserves further investigation.

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P166

Teriparatide in the treatment of chronic postoperative hypoparathyroidism in two hemodialysis patients

Anna Eremkina, Anna Gorbacheva, Valeria Enenko, Natalia Mokrysheva
Endocrinology Research Center, Parathyroid disorders department, Moscow, Russia C.I.S.

Background/Introduction: The surgery for secondary CKD-associated hyperparathyroidism can lead to persistent hypoparathyroidism. Prescribing of vitamin D and calcium supplements is not always sufficient. In hemodialysis patients with hypoparathyroidism and osteoporosis the administration of teriparatide is an option, but the data is strictly limited.

Purpose: To describe the two hemodialysis patients with chronic postoperative hypoparathyroidism treated with teriparatide.

Methods: Patient A: 56 y.o. female. Standard therapy with alfacalcidol (0,25 mcg/day) and calcium carbonate (3000 mg/day) was followed by hypocalcemia (2.07-2,11 mmol/l) and hyperphosphatemia (1,8-2.23 mmol/l). DEXA showed severe osteoporosis in radius (T_{score} -5.1SD) and spine (T_{score} -3.2SD), osteopenia in hip (T_{score} -1.7SD). No vertebral fractures were found.

Patient B: 41 y.o. female. Lab tests revealed hypocalcemia (1.76 mmol/l), normophosphatemia (0.9 mmol/l) on alfacalcidol (2 mcg/day) and calcium carbonate (3000 mg/day). BMD was reduced in radius (Z_{score} -3.3SD), in spine (Z_{score} -1.5SD) and in hip (Z_{score}

-2.3SD). There were several vertebral fractures (Th7-Th10). She had other low-trauma fractures and also underwent bilateral endoprosthetics of the hip joints.

Both patients were prescribed teriparatide 20 mcg daily.

Results: Patient A: On combined therapy with teriparatide, alfacalcidol, sevelamer and calcium carbonate normocalcemia and normophosphatemia were achieved. Over 16 months of treatment, BMD was increased in spine (T_{score} -2.1SD) and hip (T_{score} -1.4SD) and slightly decreased in radius (T_{score} -5.4SD). Patient didn't develop new fractures.

Patient B: Serum calcium and phosphorus levels remained within reference ranges during 17-months teriparatide. The patient continued to take alfacalcidol and calcium carbonate in smaller doses. DEXA scan showed a significant BMD growth in spine (Z_{score} 3.8SD) but it could be related to the new vertebral fractures. There was also an increase in the radius BMD (Z_{score} -1.9SD).

Conclusion(s): In dialysis patients with hypoparathyroidism teriparatide may help to achieve the normocalcemia and to improve the BMD in some cases. However, further research is needed.

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P167

Bone mineral density in postmenopausal women with type 2 diabetes mellitus with and without diabetic nephropathy

Simeon Monov[a], Russka Shumnalieva[a], Daniela Monova[b]
[a]*Medical University - Sofia, Department of Rheumatology, Sofia, Bulgaria*
[b]*Medical University - Sofia- Medical Institute - MVR, Department of Internal Medicine, Sofia, Bulgaria*

Background/Introduction: Diabetes mellitus and osteoporosis are both common human diseases. Diabetic nephropathy is characterized by the presence of pathological quantities of urine albumin excretion, diabetic glomerular lesions, and loss of glomerular filtration rate in diabetics. Little evidence has been reported on relationships between BMD and albuminuria.

Purpose: To compare the bone mineral density (BMD) in postmenopausal women with type 2 diabetes mellitus (T2DM) with and without diabethic nephropathy.

Methods: We retrospectively analysed the BMD of the lumbar spine and femur using dual-energy X-ray absorptiometry in 84 postmenopausal women with T2DM with (39) and without (45) diabetic nephropathy. The serum levels of calcium, phosphorus, total alkaline phosphatase, and urine albumin excretion were measured in all participants. Diagnosis of albuminuria was based on albumin-creatinine ratio (ACR).

Results: Age, body mass index (BMI) and time since menopause were not significantly different between the two groups. The T-scores of basal BMD at L4 were significantly lower in patients with diabethic nephropathy ($-0{,}94 \pm 0{,}40$) compared to patients without nephropathy. No significant differences in serum creatinine were detected between two groups of patients. Our data suggest that ACR was negatively associated with lumbar spine and femoral neck BMD.

Conclusion(s): Our results suggest that postmenopausal women with diabetic nephropathy have a lower BMD and are at increased risk of osteoporosis in the lumbar spine compared with postmenopausal women without diabethic nephropathy. ACR was negatively associated with lumbar spine and femur neck BMD. One of the explanations that has been proposed for the association between albuminuria and osteoporosis is that albuminuria is associated with reduced bone blood flow, resulting in a decreased rate of bone remodeling and the development of osteoporosis.

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P170

How the duration of energy deficit in mice model affects the body composition, bone phenotype, and bone marrow stromal cell differentiation capacity?

Viktorija Avilkina[a], Damien Leterme[a], Guillaume Falgayrac[b], Pierre Marchandise[b], Flore Miellot[a], Véronique Gauthier[a], Christophe Chauveau[a], Olfa Ghali Mhenni[a]
[a]*MAB Lab EA4490, Université du Littoral-Côte d'Opale, Boulogne sur Mer, France*
[b]*MAB Lab EA4490, Univ Lille Nord de France, Lille, France*

Background/Introduction: Anorexia nervosa is known to induce changes in bone parameters that depend on the duration and seriousness of the disease. Many studies using mouse models showed that bone parameters are altered in response to extended caloric restriction (CR) as described in anorectic patients. Sirtuin Type 1 (Sirt1), a histone deacetylase (HDAC), was shown to have pro-osteoblastic and anti-adipogenic effects by deacetylating transcription factors. We recently demonstrated that a 10-week Separation-Based Anorexia (SBA) protocol, which causes a 18% body weight loss and bone alterations, is associated with a significant decreased Sirt1 expression in Bone Marrow Stromal Cells (BMSCs) from female mice.

Purpose: Thus, we hypothesised that this decrease is involved in changing BMSC differentiation capacity and therefore inducing bone alterations.

Methods: To test this hypothesis, we compared SBA mice after 10 and 4 weeks of protocol, to determine if alterations of bone, BMSC differentiation and Sirt1 expression levels could be disconnected.

Results: Concerning body composition, 4-week and 10-week SBA protocols induced a significant decrease in subcutaneous and visceral adipose tissues as well as a shortened tibia length. Analysis of bone parameters showed that these two SBA protocols led to a reduction in BV/TV and trabecular thickness. However, a significant decrease in cortical thickness is only observed in 10-week protocol. Interestingly, only the 10-week SBA protocol induced a significant and lasting decline in Sirt1 mRNA level, accompanied with an increase in adipogenesis at the expense of osteogenesis.

Conclusion(s): In conclusion, our study showed that energy deficit duration is involved in changes in body composition, bone parameters, BMSC differentiation and Sirt1 expression. It also showed that BMSC differentiation and cortical bone phenotype were associated to Sirt1 expression. The prospects are now to determine the molecular mechanisms responsible for decreased Sirt1 expression by epigenetic and RNA sequencing approaches.

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P173

Zebrafish as a model to assess the effects of thalidomide in limb development

Débora Varela[a,b], Tatiana Varela[a,b], Joana Rosa[a], Ana Nunes[a], Natércia Conceição[a,b], M. Leonor Cancela[a,b,c,d]
[a]*University of Algarve, Centre of Marine Sciences CCMAR, Faro, Portugal*

[b]University of Algarve, Faculty of Medicine and Biomedical Sciences FMBS, Faro, Portugal
[c]University of Algarve, Centre for Biomedical Research CBMR, Faro, Portugal
[d]University of Algarve, Algarve Biomedical Centre ABC, Faro, Portugal

Background/Introduction: Thalidomide is a pharmaceutical compound that became popular during the 1950s-60s as treatment for morning sickness usually prescribed to pregnant woman. However, cases of birth defect involving shortened or absent limbs were detected in babies whose mothers took thalidomide during the first weeks of pregnancy. The use of thalidomide also causes multiple defects such as cardiac malformations. The phenotype observed in these newborn babies resembles the observed in Holt-Oram syndrome (HOS) patients. HOS is a rare congenital condition characterized by malformations in the upper limbs and congenital cardiac defects, caused by mutations in the *TBX5* gene. *TBX5* gene encodes a transcription factor that plays a role in heart and skeletal development.

Purpose: Given the similarities in phenotypes between thalidomide affected babies and those born with a mutation in the *TBX5* gene, the aim of this work was to analyze the effect of thalidomide in zebrafish both at phenotype level and in the pathways regulated by TBX5.

Methods: To achieve this goal, zebrafish eggs were exposed to 400 μM of a thalidomide solution. After three days of treatment, morphological changes in zebrafish larvae were visualized using a stereomicroscope. Whole larvae were also collected for RNA extraction to assess the levels of expression of genes known to be part of the TBX5 signaling pathway.

Results: Our results showed that 75% of the larvae exposed to thalidomide presented either a complete absence or less developed pectoral fins. Gene expression analysis showed that *sall4* and *lef1* were up-regulated while *fgf10a* was down-regulated in zebrafish exposed to thalidomide indicating that the pathways regulated by TBX5 are affected by this drug.

Conclusion(s): In conclusion, our work confirmed that, upon treatment with thalidomide, zebrafish develop morphological malformations similar to those observed in humans thus indicating that it can be a valid model to investigate the molecular pathways altered by thalidomide treatment.

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P174
Screening for osteoporosis in pediatric patients with Glass syndrome is strongly recommended

Anne Dittrich, Jos Draaisma
Radboudumc Amalia Children's hospital, Pediatrics, Nijmegen, Netherlands

Background/Introduction: SATB2-associated syndrome (SAS), or Glass syndrome, is caused by mutations affecting the special AT-rich sequence binding protein 2 (SATB2). The syndrome is characterized by intellectual disability with severely limited speech, high palate and dentofacial abnormalities. Abnormal bone mineralization in SAS has been reported previously. There are no guidelines for the prevention and therapy of osteoporosis in these patients.

Purpose: To propose regular screening for osteoporosis in patients with SAS, because treatment might be necessary.

Methods: Clinical description of three patients and review of literature.

Results: Case 1 is a 12 year old boy with SAS, 2 bone fractures in history. Dual-energy X-ray absorptiometry (DXA) showed a low bone density of de lumbar spine (Z score -3.5) and right hip (Z-score -3.3). He was started on pamidronic acid four times a year.

Case 2 is a 6 year old girl with SAS. Her DXA scan showed a low low bone density of de lumbar spine (Z-score -1.7). She never had a fracture.

Case 3 is a 8 year old boy with SAS. He had never bone fractures. His DXA scan showed normal bone mineral density.

Laboratory results of all three patients showed high alkaline phosphatase.

In literature, these diversity in bone density in SAS patients is also described.

Conclusion(s): Our case reports show the difference in bone density with clinical relevance in SAS patients, varying from low bone density with bone fractures, to normal bone density. This highlights the need of active screening on bone density in children with known SAS or undiagnosed patients with a history of intellectual disability in combination with severely limited speech and fractures, scoliosis and/or tibial bowing. Treatment might be necessary.

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P175
Osteogenesis Imperfecta in a girl with c.441 of the 2 heterozygous dominant frameshift variant gene of COL A1

Liliana Mejia Zapata[a,b]
[a]Fundation Clinica Infantil Club Noel, endocrinology paediatric, Cali, Colombia
[b]Universidad Libre GRIMPED, pediatric, Cali, Colombia

Background/Introduction: Osteogenesis Imperfecta (OI) is a genetic condition characterized by alterations in collagen formation leading to increased bone fragility and fractures. Clinical presentation ranges from mild cases to severe ones with multiple fractures including in utero, perinatal death, progressive bone deformity and short stature. Dentinogenesis imperfecta, blue or grey sclera, hearing loss, articular and skin hypermobility can be present as well. 80 to 90% of cases are due to mutations in one of the genes which encodes procollagen chain 1: pro-α1 (gene COL1A1) and pro α2 (gene COL1A2). There are several inheritance patterns. Genetic alterations of collagen metabolism include post-translational modifications affecting the transport to the extracellular space, the folding and assembly of collagen fibers, mineralization, maturation of osteoblasts. Management of OI is multidisciplinary.

Purpose: To present a patient with Osteogenesis Imperfecta (OI) carrying a mutations of frameshift variant COL1A1 and COL1A2 genes.

Methods: We investigated the pathogenic mutations **gene COL1A1 and COL1A2** and analyzed their relationship with the phenotype in the patient with OI using next-generation sequencing (NGS).

Results: A five-year-old girl with father and grandfather with OI and multiple fractures. Weight and stature within 2 SD, no fractures, blue sclera, no hearing loss, no bone deformities. normal blood lab results: Serum calcium 10.5mg/ dl , 25-(OH) D 61ng /ml, PTH 13 pg/ml, alkaline phosphatase 213 UI/L, serum phosphate 4.5 mg/dl. Normal bone X-rays and renal ultrasound. Genetic studies revealed two OI variants in: Gene COL1A1 and Col1A2, splice homozygous recessive variant of gene COLA2 and gene COLA1 c.441 of 2 dominant frameshift variant. His father has a variant c.441del C. in this gene COL1A1 and homozygous variant c.937-3c>t y c.1645c>g. in this gene COL1A2.

Conclusion(s): Probably the patient did not fracture because the presence of the two variants conferred some degree of collagen quality. This mutation has not been described in Colombia.

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P177

Evaluation of bone health in pediatric patients with chronic anticoagulation pharmacotherapy

Adalbert Raimann[a], Janina Patsch[b], Michael Weber[b], Florentina Haufler[a], Christiane Pees[c], Sulaima Albinni[c], Christoph Male[c], Katharina Thom[c]
[a]*Medical University of Vienna, Division of Pediatric Pulmonology-Allergology and Endocrinology- Comprehensive Center for Pediatrics-Department of Pediatrics and Adolescent Medicine, Vienna, Austria*
[b]*Medical University of Vienna, Department of Biomedical Imaging and Image-Guided Therapy, Vienna, Austria*
[c]*Medical University of Vienna, Division of Pediatric Cardiology and Haemostaseology, Vienna, Austria*

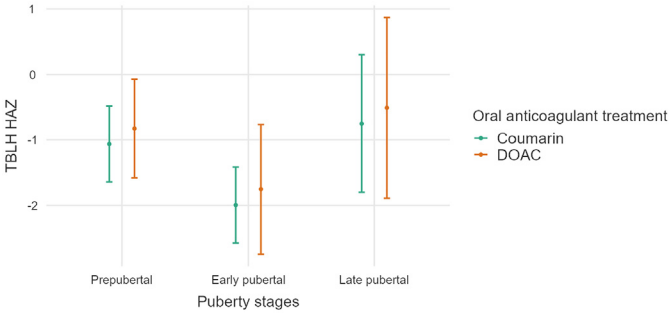
Background/Introduction: Vitamin K deficiency, as demonstrated by levels of undercarboxylated osteocalcin has been shown an independent predictor of osteoporosis and hip fracture. While the importance of Vitamin K for skeletal health in adults has been demonstrated, data on bone health in children with chronic oral anticoagulation treatment (OAT) are sparse. Effects on bone metabolism in the vulnerable phase of linear bone growth remain poorly understood.

Purpose: This study aimed to:

1) assess BMD in children with chronic diseases under OATs.
2) identify associations of potentially modifiable risk factors of low BMD.

Methods: Bone densitometry was assessed cross-sectionally using Hologic QDR4500-Elite densitometer and corrected for age and height. Biochemical assessment of bone parameters was performed on an automated IDS-iSYS analyzer.

Results: 39 patients with OAT (4-18 years; 12 females) have been included. Mean BMD was decreased for both lumbar spine (LS; -0.7SDS) and total body less head (TBLH; -1.32SDS). Especially early pubertal stages were associated with lower BMD TBLH (p=0.01). BMI LS correlated significantly with BMI SDS (R^2 0.24; p=0.003). Neither type of OAT, treatment duration or INR target affected BMD. 25.6% of patients revealed Vitamin-D deficiency, with significantly lower values after onset of puberty (p=0.03).



Conclusion(s): Pediatric patients under OAT revealed a substantially reduced BMD. Whilst choice of OAT was not associated with BMD alterations, low BMI and delayed progression of puberty represent important risk factors. Awareness of this potential treatment options as well as of high rates of Vitamin D deficiency especially in pubertal patients could substantially contribute to improve bone health in this vulnerable patient group.

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P180

Novel heterozygous mutations in gene SERPINH1 cause autosomal recessive osteogenesis imperfecta type X

Liliana Mejia Zapata
Fundación Clinica Valle del Lili y Clinica Infantil Club Noel, Valle de Cauca, Cali, Colombia

Background/Introduction: Osteogenesis imperfecta (OI) is a heritable bone disorder characterized by low bone mineral density, recurrent fractures, and progressive bone deformities. The gene (SERPINH1) Serpine peptidase inhibitor clade H, member 1, encodes a member of the serpin superfamily of serine proteinase inhibitors. The encoded protein is localized to the endoplasmic reticulum and plays a role in collagen biosynthesis as a collagen-specific molecular chaperone. Mutation in gene SERPINH1 , which encodes heat shock protein 47 (HSP47), leads to rare autosomal recessive OI type X.

Purpose: Describe a pediatric patient with a new mutation for osteogenesis imperfect

Methods: We investigated the pathogenic mutations **del gene** SERPINH1 and analyzed their relationship with the phenotype in the patient with OI using next generation sequencing NGS

Results: The patient is a 12 year-old female,with blue scleras, dentinogenesis imperfecta,no hearing loss ,suffered from multiple fractures, low bone mass, and bone deformities in the femur and scoliosis. Heterozygous variants were found in SERPINH1 gene as follows: c.1228A>G (plys 410 Glu). Management with bisphosphonates was useful in increasing BMD Z score and reduced bone fracture risk in this patient.

Conclusion(s): In our knowledge, we reported novel heterozygous mutations in SERPINH1 in a Colombian OI patient for the first time, which suggests the spectrum of phenotype and genotype of rare OI type X.

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P181

Acute left heart failure in a McCune-Albright syndrome patient with negative preoperative cardiovascular system screening after proximal femur osteotomy – a case report

Guangtao Fu
Guangdong Provincial People's Hospital, Orthopedics, Guangzhou, China

Background/Introduction: McCune-Albright syndrome (MAS) is a rare genetic disease characterized by polyostotic fibrous dysplasia, skin hyperpigmentation, and endocrine dysfunction. Due to severe deformity of lower limbs caused by fibrous dysplasia in proximal femur or tibial, substantial numbers of patients diagnosed with MAS admitted to orthopedics clinics for surgical interventions.

Purpose: Although the skeletal and endocrine abnormalities of MAS is well recognized by most clinicians, a rare but fatal event of MAS, cardiovascular complication, was seldomly reported.

Methods: An 18-year-old female who was suffering from left lower limb deformity and thigh pain for more than 10 years admitted to our center recently. She was diagnosed with polyostotic fibrous dysplasia according to preoperative examination and underwent proximal femur osteotomy for deformity correction. Unfortunately, she developed acute left heart failure in the 1st

postoperative day under the condition of normal preoperative echocardiography and electrocardiogram screening, and the limited postoperative fluid replacement volume. Noninvasive positive-pressure ventilation, diuresis, and anti-infection therapy were administrated immediately, and all the symptoms were significantly relieved in the second day morning.

Results: The patient was finally diagnosed with McCune-Albright syndrome according to the genetic test of the pathological bone tissue in proximal left femur, which revealed the mutation of c.601C>T. It was proposed that the mutation of Gs would cause overproduction of cAMP and enhanced phosphorylation of calcium channel, which subsequently increased the permeability to Ca^{2+} of myocardial cells membrane. As a result of intracellular Ca^{2+} accumulation, cardiac myocytes were continuously overactivated and cause increased heart rate and myocardial contractility, which eventually induced cardiac dysfunction.

Conclusion(s): In the present study, the cardiovascular system involvement of MAS might be a reasonable explanation of the former-mentioned phenomenon. We believed that this case report would help orthopedic surgeons in making clinical decisions and prognosis assessment for patients with MAS before surgical intervention.

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P182

Online education improves competence in identifying fibrodysplasia ossificans progressiva (FOP)

Christy Rohani-Montez[a], Sarah Waterhouse[a], Richard Keen[b]
[a]*Medscape Education, Global, London, United Kingdom*
[b]*Royal National Orthopaedic Hospital, Metabolic Bone Disease Centre, Stanmore- Middlesex, United Kingdom*

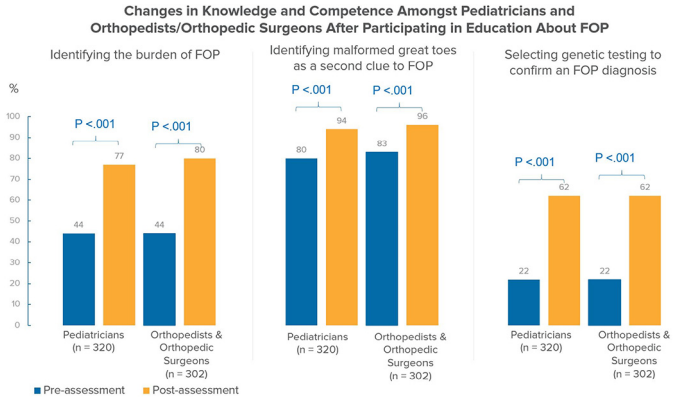
Background/Introduction: As an ultra-rare disease, FOP misdiagnosis approaches 90% worldwide. Flare-ups are often mistaken for tumors and biopsied, exacerbating progression, and causing great harm to the patient. Clinicians need awareness of the burden of FOP and how to identify it earlier.

Purpose: This study was conducted to determine whether online text-based independent medical education could improve clinicians' knowledge and competence in identifying key features of FOP and confirming a diagnosis.

Methods: Pediatricians and orthopedists/orthopedic surgeons participated in a text-based activity and completed pre- and post-questions.[1] The effects of the education on knowledge and competence were assessed using a 3-question, repeated pairs, pre-assessment/post-assessment study design. For all questions combined, the chi-square test assessed differences from pre- to post-assessment. P values <.05 are statistically significant. The activity launched on October 1, 2020, and data were collected through December 11, 2020.

Results: Overall significant improvements were seen after participation for pediatricians (49% average correct response rate at pre-assessment vs 77% at post-assessment; P<.001, N=320), and orthopedists/orthopedic surgeons (50% average correct response rate at pre-assessment vs 79% at post-assessment; P<.001, N=302). Significant improvements were observed in clinicians' knowledge of FOP burden, and competence in identifying key features and confirming a definitive diagnosis (figure).

After participating, 77% of pediatricians and 74% of orthopedists/orthopedic surgeons had measurable improved confidence related to identifying signs and symptoms suggestive of FOP.



Conclusion(s): This study demonstrates the success of online, text-based education in improving clinicians' knowledge competence in identifying FOP. This could lead to improved overall outcomes for these patients.

Reference: 1. Keen R. *Check the Toes Before Biopsy!* Launched: 10/1/2020. Data as of 12/11/2020. Available at www.medscape.org/viewarticle/935832

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P184

Vertebral fracture assessment in post-surgical hypoparathyroidism

Cristiana Cipriani[a], Salvatore Minisola[a], John Bilezikian[b], Diacinti Davide[c], Colangelo Luciano[a], Piazzolla Valentina[a], Angelozzi Maurizio[a], Nieddu Luciano[d], Pepe Jessica[a], Diacinti Daniele[e]
[a]*Sapienza Università di Roma, Clinical- Internal- Anesthesiological and Cardiovascular Sciences, Rome, Italy*
[b]*Columbia University, Division of Endocrinology, New York, United States*
[c]*Sapienza Università di Roma, Oral and Maxillo-Facial Sciences, Rome, Italy*
[d]*UNINT University, Faculty of Economics, Rome, Italy*
[e]*Sapienza Università di Roma, Radiological Sciences- Oncology and Pathology, Rome, Italy*

Background/Introduction: There are no definitive data on fracture risk, particularly vertebral fractures (VF), in hypoparathyroidism.

Purpose: We evaluated the prevalence of VF assessed by Vertebral Fracture Assessment (VFA) in postmenopausal women with chronic post-surgical hypoparathyroidism.

Methods: We studied 45 postmenopausal women (mean age 66±8 years) with chronic post-surgical hypoparathyroidism and 40 age-matched healthy postmenopausal women (64±8.6). In all subjects, lumbar spine (L1-L4), femoral neck and total hip BMD were measured by dual X-ray absorptiometry (DXA) (Hologic Inc., USA). The site-matched spine Trabecular Bone Score (TBS) was calculated by the TBS iNsight software (Medimaps, Switzerland). Assessment of VF was made by VFA (iDXA, Lunar GE, USA) using the semiquantitative method and the algorithm-based qualitative assessment (ABQ) for mild vertebral deformities. A radiologist specialized in metabolic bone disease reviewed VFA exams and manually corrected position of the marker when necessary, according to Hurxthal criteria and reviewed the VFA report according to the ABQ method.

Results: All-sites mean BMD values were higher in the hypoparathyroid vs the control group, while TBS was not significantly different (Table 1). By VFA, we observed a 15.5% prevalence of VF in hypoparathyroid vs 7.5% in healthy subjects. Hypoparathyroid women with VF had symptoms of hypoparathyroidism in 57% of cases, while those without VF had symptoms in 31% of cases. The logistic regression analysis showed a trend towards a significant independent association of VF with FRAX® without neck BMD values for major fractures (OR=1.11; p = 0.09) and for hip fractures (OR=1.27; p = 0.06).

Table 1. Characteristics (mean values ± SD) of the hypoparathyroid and the healthy women

Characteristics	Hypoparathyroid women (n= 45)	Healthy women (n= 40)	p value*
Age, years	66 ± 8	64.2 ± 8.6	NS
Height, cm	160 ± 7	160 ± 5.9	NS
Weight, kg	68.3 ± 10.9	66.9 ± 9.5	NS
BMI, kg/m²	26.7 ± 4.3	26.3 ± 4.3	NS
Time since menopause, years	16 ± 8	14.8 ± 9.8	NS
Time since diagnosis, years	19 ± 10.9	-	-
Symptoms, n (%)	18 (40%)	-	
Neuromuscular	18 (40%)	-	-
Neuropsychiatric	2 (4.4%)	-	
Serum total calcium, mg/dL (nr 8.4 - 10)	8.4 ± 0.3	9.6 ± 0.4	<0.0001
Serum PTH, pg/mL (nr 6.5 - 36.6)	7 ± 4.2	26.5 ± 6.6	<0.0001
L1-L4 aBMD, g/m²	1.028 ± 0.163	0.945 ± 0.126	0.05
T-score	-0.3 ± 1.5	-0.9 ± 1	0.06
Femoral Neck aBMD, g/m²	0.960 ± 1.327	0.703 ± 0.1	<0.05
T-score	-0.8 ± 1.1	-1.4 ± 0.9	<0.02
Total Hip aBMD, g/m²	0.916 ± 0.143	0.842 ± 0.104	<0.02
T-score	-0.2 ± 1.2	-0.8 ± 0.8	<0.05
TBS	1.19 ± 0.12	1.16 ± 0.17	NS
FRAX® without Neck BMD (%)			
Major fractures	8.7 ± 5.7	6.8 ± 4.4	0.06
Hip fractures	2.9± 2.9	2.1± 2.4	0.06
FRAX® with Neck BMD (%)			
Major fractures	6.4 ± 3	6.1 ± 3.3	NS
Hip fractures	1.2 ± 1.2	1.5 ± 1.4	NS

Conclusion(s): We report for the first time data on the use of VFA in postsurgical hypoparathyroidism. Our data demostrate a clinically significant prevalence of VF in this cohort despite normal BMD values. Clinical risk factors for fractures seem to be associated with the presence of VF in postmenopausal women with chronic hypoparathyroidism.

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P185

Giant parathyroid adenoma miming a neck cancer associated with primary hyperparathyroidism and complete cystic transformation

Mara Carsote[a], Eugenia Petrova[b], Dumitru Ioachim[c], Anda Dumitrascu[d], Alexandra Ioana Trandafir[e], Ana Valea[f], Nicoleta Dumitru[b], Cristina Corneci[g], Mariana Ileana Greere[h], Gabriela Voicu[i], Mircea Ghemigian[j], Adina Ghemigian[b]

[a]*C.I.Parhon National Institute of Endocrinology & C.Davila University of Medicine and Pharmacy- Bucharest- Romania, Endocrinology, Bucharest, Romania*

[b]*C.I.Parhon National Institute of Endocrinology & C.Davila University of Medicine and Pharmacy, Endocrinology, Bucharest, Romania*

[c]*C.I.Parhon National Institute of Endocrinology, Pathology, Bucharest, Romania*

[d]*C.I.Parhon National Institute of Endocrinology, Imaging & Radiology, Bucharest, Romania*

[e]*C.I.Parhon National Institute of Endocrinology, Endocrinology, Bucharest, Romania*

[f]*I. Hatieganu University of Medicine and Pharmacy & Clinical County Hospital, Endocrinology, Cluj-Napoca, Romania*

[g]*C.I.Parhon National Institute of Endocrinology, Anesthesia & Perioperative Care, Bucharest, Romania*

[h]*C.I.Parhon National Institute of Endocrinology, Anestesia & Perioperative Care, Bucharest, Romania*

[i]*C.I.Parhon National Institute of Endocrinology, Radiology- DXA, Bucharest, Romania*

[j]*C.I.Parhon National Institute of Endocrinology, Surgery, Bucharest, Romania*

Background/Introduction: Giant parathyroid adenoma (GPTA) is an exceptional distinct entity, either functional - associated with primary hyperparathyroidism (PHP), or not. Complete cystic transformation is even rarer.

Purpose: We introduce such case of cystic GPTA-PHP with large dimensions that was first admitted as a neck (thyroid) cancer with compressive symptoms.

Methods: Case report. Patient' consent was obtained(July2020)

Results: A 58-year male admitted for acute neck compression (progressive within last 3 months) associates a large right-anterior cervical mass (with gradual increase for the last 2 years, without investigations). Family and personal medical history: negative.

Biochemistry: PHP based on total calcium (TCa)=12.9mg/dL(N:8.4-10.2) and PTH=548pg/mL(N;15-65). Also: 25OHD=17ng/mL(N<30), phosphorus=2.13mg/dL(N:2.5-4.5), 24-h urinaryCa=0.46g/24h (N:0.07-0.3), and high bone turnover markers like CrossLaps=2.8ng/mL(N:0.104-0.504), osteocalcin=253ng/mL(N:14-46), alkaline phosphatase=209U/L(N:38-129); negative assays for MEN2A.

Other investigations: mild renal insufficiency, no kidney stones, normal thyroid assessment, osteoporosis based on DXA: 1/3distal radius bone mineral density (BMD)=0.444g/sqcm, T-score=-4.5SD, Z-score=-4.1SD, lumbarL1-4 BMD=0.857g/sqcm, T-score=-3SD, Z=-2.2SD, Trabecular Bone Score of 1,174.

Computed tomography shows well-shaped, encapsulated, right cervical tumor, with mass effect on thyroid, esophagus, sternocleidomastoid muscle, and trachea, of 9.18X7.64cm, with cystic consistence, negative contrast uptake at liquid area.

After open neck surgery and tumor removal, pathological report reveals a superior right giant adenoma PTA with total cystic

transformation, of 9.5X7cm, weight=117grams (with liquid), =40grams (without liquid), wall thickness=0.3-0.7cm (with principal cells). Post-operatory symptomatic hypocalcemiaTCa=8.4mg/dL (N:8.5-10.2) with normal PTH=39pg/mL(N:15-65).

Conclusion(s): Particular aspect of the case: the rarity of GPTA of such dimensions, with cystic appearance. GPTA is functional but the presentation as an emergency is mostly related to the presence of a large neck tumor rather than PHP complicated with de novo osteoporosis. Complete cystic transformation probably follows the secretory activity of solid adenoma. The hemorrhagic shift seems spontaneous. Parathyroid tumors with rapid evolution and mass effect need to be differentiated from parathyroid carcinoma.

Key words: giant parathyroid adenoma, cyst, primary hyperparathyroidism, tumor, hypercalcemia

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P187

Arnold Chiari malformation (ACM) in XLH: Rare but important complication of a rare disease

Zaineb Mohsin, Muhammad Kassim Javaid
Nuffield Orthopaedics Centre, Rheumatology, London, United Kingdom

Background/Introduction: X Linked hypophosphataemia (XLH) is a rare condition that typically causes musculoskeletal manifestations in adults. However, it is associated with serious neurological manifestations that are under recognized such as the Arnold-Chiari malformation.

Purpose: To highlight the need for a high index of suspicion for ACM which can have non-specific clinical features but can lead to considerable morbidity and mortality.

Methods: A 29 year old gentleman had severe X-Linked Hypophosphataemia. He was wheel-chair enabled from severe hip and back pain despite extensive orthopaedic interventions. An MRI of the c-spine at the age of 23 for severe headaches/ migraines, when he was diagnosed as suffering from an Arnold Chiari malformation and he was under annual neurosurgical follow up. He was diagnosed with obstructive sleep apnoea, attributed to his BMI of 36. At the age of 27, he was admitted to ITU with respiratory failure and pneumonia. Post discharge, he complained of dysphagia and diagnosed with bronchiectasis. Bilateral neuropathy of his hands was attributed to a post ITU syndrome.

Results: He was readmitted to ITU the following year for respiratory failure and septic shock presumed and unfortunately succumbed to his illness. The unifying diagnosis for his sleep apnoea, aspiration with secondary bronchiectasis, dysphagia and respiratory failure is ACM.

Conclusion(s): ACM in XLH could be explained by calvarial thickening, decreased size of the posterior fossa and sagittal synostosis. A neurologic cause should be considered for adults with XLH who develop respiratory or swallowing symptoms, even in the absence of prominent signs and symptoms. MRI is crucial in diagnosing ACM. Screening questions for ACM condition should be part of the routine followup checklist.

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P189

A rare case of thoracic Gorham-Stout disease presenting with recurrent pleural effusions

Zaineb Mohsin[a], Karen Partington[b], James Teh[b], Najib Rahman[c], Muhammad Kassim Javaid[a]
[a]*Nuffield Orthopaedics Centre, Rheumatology, London, United Kingdom*
[b]*Oxford University Hospitals NHS Trust, Radiology, London, United Kingdom*
[c]*Oxford University Hospitals NHS Trust, Respiratory Diseases, London, United Kingdom*

Background/Introduction: Gorham-Stout disease (GSD) is a rare complex lymphatic anomaly characterized by osteolysis. Thoracic involvement can be associated with a chylothorax, a poor prognostic sign with a mortality range of 33 to 53%.

Purpose: To present the clinical features of this disease when it affects the thorax and to highlight the importance of collaboration with international experts.

Methods: A 44-year-old woman gave a 10-year history of intermittent severe musculoskeletal pain of the neck and right shoulder lasting 1 to 4 weeks. Following investigations by orthopaedics for shoulder pain and neurology for neck pain, she was referred to complex pain clinics. She developed breathlessness with the pain attacks in 2020 and a chest x-ray showed a moderate right pleural effusion with incidental finding of missing right ribs 2-5. An MRI demonstrated C7, T1 and T2 and sternal involvement. Despite drainage of the pleural effusion, follow up imaging revealed re-accumulation of pleural fluid and minimal pericardial and left sided pleural effusions. The CA-125 was raised and extensive gynaecological investigations exclude malignancy. Bone profile including PINP, liver and renal function were normal. Pleural fluid was exudative without a chylothorax. The right pleural histopathology showed non-specific inflammation and mild fibrosis with negative immunohistochemistry for lymphatic vessels using D2-40.

Results: Following consultation with the LGDA medical advisory board 4 weekly zoledronic acid 4mg was started with a plan to add in sirolimus to reduce mTOR activity, thought be central to the pathogenesis.

Conclusion(s): GSD is a rare bone disease that can present differently according to the anatomical region involved. Management requires a multidisciplinary approach and collaboration with international experts with a wider experience to plan effective treatment.

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P191

Physical function and mobility in adults with X-linked hypophosphatemia

Giorgio Orlando[a], Shane Clarke[b], Matthew Roy[b], Robin Lachmann[c], Jennifer Walsh[d], Marian Schini[d], Javaid Kassim[e], Alex Ireland[a]
[a]*Manchester Metropolitan University, Musculoskeletal Science and Sports Medicine Research Centre- Department of Life Sciences, Manchester, United Kingdom*
[b]*University Hospitals Bristol, Department of Rheumatology, Bristol, United Kingdom*
[c]*Charles Dent Metabolic Unit, University College London Hospitals, London, United Kingdom*
[d]*University of Sheffield, Department of Oncology and Metabolism, Sheffield, United Kingdom*
[e]*University of Oxford, Nuffield Department of Orthopaedics- Rheumatology and Musculoskeletal Sciences, Oxford, United Kingdom*

Background/Introduction: X-linked Hypophosphatemia (XLH) is a rare genetic disorder affecting phosphate metabolism. Whilst muscle weakness has been reported in adults with XLH, there is little data describing detailed physical function.

Purpose: We examined upper and lower limb function and fitness in UK adults with XLH and assessed the relationships between physical function and mobility.

Methods: Adults with XLH were recruited as part of an ongoing UK-based prospective cohort study, the RUDY Study. Participants underwent a clinical visit and physical examination. This included grip strength and jump power assessed by mechanography, six-minute walk test (6MWT) and short physical performance battery (SPPB). Scores were compared with existing age and sex-specific normative data using t-test, whereas correlations among outcomes were processed using Pearson's correlation coefficient.

Results: Twenty-nine adults with XLH (15 males and 14 females), with a mean age of 46.8±15.8 years were enrolled to the study. Grip strength was 27% lower ($p=0.005$) and jump power 63% lower in individuals with XLH than normative values ($p<0.0001$), with greater deficits evident in the lower than upper body ($p=0.003$). Aerobic fitness was 42% lower in XLH individuals when compared to reference values ($p<0.0001$). Mean SPPB score was 8.6±3.3, with 14/29 individuals having a score of <10 indicating impaired mobility. Univariate correlations revealed that handgrip strength ($r=0.591$, $p<0.001$), jump power ($r=0.630$, $p<0.001$) and aerobic fitness ($r=0.739$, $p<0.0001$) were all highly correlated to mobility (SPPB).

Conclusion(s): Adults with XLH had weaker lower body power than other components of physical function. Upper and lower limb function and aerobic fitness were all strongly associated with impaired mobility in this population, which suggests that the origin of mobility deficits may be multifactorial. Further studies are required to understand underlying mechanisms, and to develop novel treatment approaches to improve physical function and mobility.

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P193

Development of an observational registry for genetic hypophosphatemia and acquired renal phosphate wasting in The Netherlands: ORPHOS-NED

Ariadne Bosman[a], Natasha Appelman-Dijkstra[b], Joop van den Bergh[c], Arend Bökenkamp[d], Annemieke Boot[e], Martin de Borst[f], Bram van der Eerden[a], M. Carola Zillikens[a]

[a]*Erasmus MC- University Medical Center Rotterdam, Department of Internal Medicine, Rotterdam, Netherlands*

[b]*Leiden University Medical Center, Department of Internal Medicine, Leiden, Netherlands*

[c]*VieCuri Medical Center, Department of Internal Medicine, Venlo, Netherlands*

[d]*Emma Children's Hospital- Amsterdam University Medical Center- Vrije Universiteit, Department of Pediatric Nephrology, Amsterdam, Netherlands*

[e]*University Medical Center Groningen, Department of Pediatrics, Groningen, Netherlands*

[f]*University Medical Center Groningen, Department of Internal Medicine, Groningen, Netherlands*

Background/Introduction: Phosphate is critical for skeletal development and mineral metabolism. Phosphate deficiency leads to e.g. muscle weakness and rickets or osteomalacia. Several inherited and acquired causes of renal phosphate wasting can lead to hypophosphatemic rickets (HR). X-linked hypophosphatemia (XLH) is the most common form of HR with an estimated prevalence of 1:20.000. The prevalence of chronic hypophosphatemia in the Netherlands and the clinical manifestations are currently unknown.

Purpose: ORPHOS-NED has been developed to identify and evaluate patients with XLH and other forms of chronic hypophosphatemia within a registry in the Netherlands.

Methods: ORPHOS-NED is a web-based registry that has been set up by a group of medical specialists, who are affiliated to the Dutch Federation of Nephrology (NFN) and the Bone Network of the Dutch Society of Endocrinology (NVE). Dutch endocrinologist and nephrologists are approached for eligible patients. Children and adults with chronic hypophosphatemia are considered for inclusion. After informed consent, a chart review is performed to collect data on several aspects of the disease: initial presentation; symptoms; radiological, genetic and laboratory examinations; treatment; and follow up. Furthermore, questionnaires are sent out to assess health-related quality of life including the Brief Fatigue Inventory, the Brief Pain Inventory, RAND36, the Health Assessment Questionnaire and the Pediatric Outcomes Data Collection Instrument.

Results: Currently, 83 pediatric and adult patients from 3 academic hospitals have been included in this registry. Inclusion of patients is ongoing. The data from this registry will lead to more insight in the prevalence, natural history, treatment and its effects on HR, quality of life, and into genotype-phenotype relations in the different genetic forms.

Conclusion(s): A Dutch nationwide registry is being set up for genetic and acquired forms of chronic hypophosphatemia, which will lead to improved insight in prevalence, causes, disease manifestations and therapy.

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P195

Activin-A induces differential gene expression exclusively in periodontal ligament fibroblasts from fibrodysplasia ossificans progressiva patients

Ton Schoenmaker[a], Michal Mokry[b], Dimitra Micha[c], Coen Netelenbos[d], Nathalie Bravenboer[e], Marjolein Gilijamse[f], Marelise Eekhoff[d], Teun de Vries[a]

[a]*ACTA, Department of Periodontology, Amsterdam, Netherlands*

[b]*UMC Utrecht, Division Laboratory- pharmacy and biomedical genetics, Utrecht, Netherlands*

[c]*Amsterdam UMC, Department of Clinical Genetics, Amsterdam, Netherlands*

[d]*Amsterdam UMC, Department of Internal Medicine section Endocrinology, Amsterdam, Netherlands*

[e]*Amsterdam UMC, Department of Clinical Chemistry, Amsterdam, Netherlands*

[f]*Amsterdam UMC, 6Department of Oral and Maxillofacial Surgery and Oral Pathology, Amsterdam, Netherlands*

Background/Introduction: Fibrodysplasia Ossificans Progressiva (FOP) is a rare genetic disease characterized by heterotopic ossification (HO). It is caused by mutations in the Activin receptor type 1 (*ACVR1*) gene resulting in enhanced responsiveness to ligands, specifically to Activin-A. Though it has been shown that capturing Activin-A protects against heterotopic ossification in animal models, the underlying mechanisms at the gene expression level causing ossifications and progression are unknown.

Purpose: Unraveling the mechanisms by which Activin-A mediates heterotopic ossification in FOP has become increasingly relevant given the promising, disease-limiting results in FOP patients in the first clinical trial with Activin-A antibodies. We investigated the transcriptomic changes induced by Activin-A of healthy control and patient-derived periodontal ligament fibroblasts (PLF) isolated from extracted teeth by RNA sequencing analysis.

Methods: To study early differences in response to Activin-A, periodontal ligament fibroblasts from 6 control teeth and from 6 FOP patient teeth were cultured for 24 hours without and with 50 ng/ml Activin-A and analyzed with RNA sequencing.

Results: Pathway analysis on genes upregulated by Activin-A in the FOP cells showed an association with pathways involved in Activin, TGFβ and BMP signaling. Gene ontology (GO) analysis using a Benjamini and Hochberg's False Discovery Rate of 5% showed an association, only in FOP cells, with GO terms that can be linked to cell adhesion, cell binding to substrate, and response to endogenous stimulus. When applying more stringent statistical criteria, differential gene expression induced by Activin-A was exclusively seen in the FOP cells. The upregulated genes with fold changes higher than 2 after 10% False Discovery Rate correction, like *SHOC2*, *TTC1*, *PAPSS2*, *DOCK7* and *LOX* are all associated with bone metabolism.

Conclusion(s): Our open ended approach to investigate the early effect of Activin-A on gene expression in control and FOP PLF shows

that the molecule exclusively induces differential gene expression in FOP cells.

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P196

Bone cell functions in PPIB knock-out mouse model for type IX osteogenesis imperfecta are distinct from classical dominant OI

Ying Liu[a], Theresa Hefferan[b], Nadja Fratzl-Zelman[c], Joan Marini[a]
[a]*National Institute of Child Health and Human Development- National Institutes of Health, Section on Heritable Disorders of Bone and Extracellular Matrix, Bethesda, United States*
[b]*Mayo Clinic, Biomaterials and Histomorphometry Core Laboratory, Rochester, United States*
[c]*Hanusch Hospital, Ludwig Boltzmann Institute of Osteology at the Hanusch Hospital of OEGK and AUVA Trauma Centre Meidling- 1st Medical Department, Vienna, Austria*

Background/Introduction: Osteogenesis imperfecta (OI) is a collagen-related bone disorder. While most OI cases are caused by dominant mutations in type I collagen, other cases are caused by recessive defects in genes encoding collagen-interacting proteins. Cyclophilin B (CyPB), encoded by *Ppib*, functions both 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.

Purpose: We reported previously that *Ppib*$^{-/-}$ mice have abnormal type I collagen post-translational modification and crosslinks. This study focuses on *Ppib*$^{-/-}$ bone cell functions.

Methods: Histomorphometry and qBEI analysis of femoral tissue, RT-PCR and alizarin red staining of osteoblasts, and *in vitro* osteoclast differentiation.

Results: Histomorphometry of *Ppib*$^{-/-}$ femora (2-month male, n=10) reveals markedly reduced cortical thickness ($p<0.0001$), reduced bone volume (BV/TV, *Ppib*$^{-/-}$ 5.8±1.9%, WT 10.4±2.7%; $p<0.001$), based on significant decrease in trabecular thickness ($p<0.05$) and number ($p<0.01$). Distinct from high turnover hypercellularity seen in dominant OI bone, *Ppib*$^{-/-}$ osteoblast number and surface are significantly decreased ($p<0.01$; $p<0.05$). TRAP-positive osteoclast number does not differ between KO and WT bone, as well as *in vitro* osteoclast differentiation. *Ppib*$^{-/-}$ mice have reduced osteoid volume (69%, $p<0.05$), MAR (37%, $p<0.0001$), and BFR/BS (37%, $p<0.01$) vs WT. CyPB deficiency in osteoblasts results in elevated matrix mineralization (Alizarin red staining, $p<0.001$), consistent with increased expression of late osteoblast differentiation markers *Sost, Mepe, Phex, Dmp1,* vs WT. Consistent with these data, qBEI analysis yielded bone mineralization density distribution with increased CaMean, CaPeak (both $p<0.001$) and CaHigh values ($p=0.014$) in femoral midshaft cortical bone of KO vs WT.

Conclusion(s): Cyclophilin B/*Ppib* KO mice, modelling type IX OI, have bone cell functions distinct from classical dominant OI with collagen defects. 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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P197

The importance of TNAP/Tnap for dental development in human cell culture and in zebrafish

Stephanie Graser[a], Sofia Paulus[a,b], Elisa Riekert[c], Franz Jakob[a], Eva Klopocki[c], Denitsa Docheva[d], Theresa Kreuzahler[a,b], Maximilian Rudert[e], Marietta Herrmann[a,b], Daniel Liedtke[c]
[a]*Bernhard-Heine-Center for Locomotion Research, Department of Orthopedics University of Würzburg, Würzburg, Germany*
[b]*IZKF Group Tissue Regeneration in Musculoskeletal Diseases, University Hospital Würzburg, Würzburg, Germany*
[c]*Institute of Human Genetics, Biocenter- University of Würzburg, Würzburg, Germany*
[d]*Experimental Trauma Surgery, Department of Trauma Surgery- University Regensburg Medical Centre, Regensburg, Germany*
[e]*Department of Orthopaedic Surgery, König-Ludwig-Haus- University of Würzburg, Würzburg, Germany*

Background/Introduction: The rare disease hypophosphatasia (HPP) is caused by mutations in the *ALPL* gene leading to a decreased activity of the ectoenzyme tissue-nonspecific alkaline phosphatase (TNAP). HPP is not only causing severe problems concerning bone mineralization but also the dental system is frequently affected. Patients lose their deciduous teeth prematurely (without tooth root resorption), the mineralization of dentin and cementum may be deteriorated, and periodontitis and caries are likely to occur.

Purpose: As the molecular mechanisms behind the dental HPP symptoms have not been completely unraveled, we aim to establish two different model systems for the analysis of TNAP's role during dental development *in vitro* and *in vivo*.

Methods: Five TNAP-deficient human telomerase reverse transcriptase periodontal ligament derived (hTERT-PDL) cell lines were established with CRISPR-Cas9. The residual *ALPL* expression was analyzed with qRT-PCR, TNAP-activity with CSPD-assay, and the differentiation capacity with qRT-PCR and Alizarin-red staining. Different staining methods, like RNA i*n-situ* hybridization and immunofluorescence for F-actin and β-catenin, have been performed to determine the function of Tnap during dental development in zebrafish embryos.

Results: Mutations were ranging from heterozygous point mutation to homozygous 48 bp deletion (leading to a premature stop codon) and their severity correlated with the residual TNAP-activity (42,52-fold +/- SD 5,82 ($p<0.0001$) reduction in homozygous deletion vs. hTERT-PDL without CRISPR/Cas9). hTERT-PDL cells with a homozygous *ALPL* mutation were, unlike the control cells, not able to differentiate. Additionally, the zebrafish was established as a new animal model system for HPP. Preliminary observations imply that blocking of Tnap function results in altered dental morphology and impaired mineralization in zebrafish.

Conclusion(s): Our experiments illustrate that both *in vitro* and *in vivo* models complement each other, provide new possibilities for answering open questions concerning the role of the TNAP/Tnap enzyme in the dental system, and support the development of additional HPP therapies in the future.

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P198

Bone tissue and osteoblasts from X-linked type XVIII OI with defects in regulated intramembrane proteolysis have distinct features

Allahdad Zarei[a], Nadja Fratzl-Zelman[b], An Dang Do[c], Megan Glassford[d], Mark Hannibal[d], Maegan Wallace[e], Paul W. Esposito[e], Kristin Lindstrom[f], Jochen Zwerina[b], Marianne Knue[c], Sara Talvacchio[c], Joan.C. Marini[g]
[a]*NICHD-NIH, Section on Heritable Disorders of Bone and Extracellular MatrixB, Bethesda, United States*
[b]*Hanusch Hospital, Ludwig Boltzmann Institute of Osteology, Vienna, Austria*
[c]*NICHD-NIH, Office of Clinical Director, Bethesda, United States*
[d]*University of Michigan Medical School, Department of Pediatrics and Communicable Diseases, Ann Arbor, United States*

[e]University of Nebraska Medical Center, Department of Orthopaedic Surgery, Omaha, United States
[f]Phoenix Childern's Hospital-Main, Division of Genetics and Metabolism, Phoenix, United States
[g]NICHD-NIH, Section on Heritable Disorders of Bone and Extracellular Matrix, Bethesda, United States

Background/Introduction: Site-2 protease (S2P), encoded by *MBTPS2*, is a Golgi transmembrane proprotein convertase of membrane-bound transcription factors, involved in cholesterol metabolism. We previously identified an X-R form of osteogenesis imperfecta (type XVIII OI) with mutations in S2P causing impaired regulated intramembrane proteolysis (RIP) of SREBP, ATF6 and OASIS, and decreased type I collagen secretion.

Purpose: We identified probands 3 and 4 with type XVIII OI: 2y4m boy with S2Pp.N459S (c.1376A>G), and 1y5m boy with novel S2P p.L455Q (c.1364T>A) mutation.

Methods: Bone and primary osteoblasts (OB) with p.N459S were investigated with qBEI, histomorphometry, qPCR, RNAseq.

Results: Male with S2P p.N459S had LE bowing on 20 wk US. He has short stature, blue sclerae, fractures of ribs, clavicles, limbs, vertebral compressions, rhizomelia of UE and LE. His L2-L4 BMD z-score < -2 (0.167 g/cm^2).

The S2P p.L455Q mutation is associated with short stature, blue sclerae, limb fractures and deformity, undertubulated long bones with LE rhizomelia, vertebral compressions, and L1-L4 DXA z-score= -7.36 (0.128 g/cm2). Total (1574 IU/L) and bone-specific (420 mcg/L) ALP and osteocalcin (68.9 ng/ml) were elevated. Oasis processing in proband FB revealed decreased 50kD S1P/S2P cleavage product.

Cortical bone from S2Pp.N459S proband had notable marrow fibrosis and was not hypermineralized, distinct from classical OI. Histomorphometry revealed increased osteoblast (14.1%; control 8.5±4.1) and osteoid surface (46.8%; control 34±6.7). *In vitro,* the S2P p.N459S mutation hampered osteoblastogenesis. Early osteoblast markers were downregulated in primary OB, whereas, late osteoblast/early osteocyte markers were upregulated. *In vitro* mineralization was severely delayed in proband OB. Transcript profiling revealed that p.N459S alters expression of genes encoding ECM constituents and involved in ECM organization.

Conclusion(s): These *MBTPS2* missense mutations support a critical role of RIP in normal bone development. The distinctive features of type XVIII OI bone tissue and OB will reveal insights into the tissue-specific mechanism of RIP.

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P200

The skeletal effect of post-natal treatment with N-acetylcysteine in a diastrophic dysplasia mouse model

Chiara Gramegna Tota[a], Chiara Paganini[a], Luca Monti[a], Giulia Quattrini[a], Antonella Forlino[a], Morgane Bourmaud[b], Martine Cohen-Solal[b], Antonio Maurizi[c], Mattia Capulli[c], Anna Teti[c], Andrea Superti-Furga[d], Antonio Rossi[a]
[a]University of Pavia, Department of Molecular Medicine- Unit of Biochemistry, Pavia, Italy
[b]University Paris, Inserm U1132 and University Paris-Diderot P7 and Department of Rheumatology- Hopital Lariboisiere, Paris, France
[c]University of L'Aquila, Department of Biotechnological and Applied Clinical Sciences, L'Aquila, Italy
[d]University of Lausanne, Department of Pediatrics- Lausanne University Hospital, Lausanne, Switzerland

Background/Introduction: Diastrophic dysplasia is a recessive skeletal dysplasia caused by mutations in the *SLC26A2* gene encoding for a sulfate/chloride antiporter of cell membrane. Functional impairment of the transporter causes reduced sulfate uptake leading to cartilage proteoglycan (PG) undersulfation. Even if intracellular sulfate is mainly dependent on extracellular uptake, a small amount comes from the catabolism of sulfur-containing amino acids and other thiols.

Purpose: Here we are investigating a treatment with N-acetylcysteine (NAC), a cysteine derivative, in dtd mice.

Methods: Mice were treated twice a day with hypodermic injections of 250 mg NAC/Kg body weight for 21 days from birth. The effect of NAC was evaluated by cartilage PG sulfation analysis, X-rays morphometry, histology of the tibia growth plate and DEXA.

Results: At the end of the treatment, cartilage PG sulfation was significantly increased in treated dtd mice compared with the placebo dtd group (84.50% vs 80.40% sulfated disaccharides, respectively; $P < 0.05$). The length of different skeletal elements (tibia, femur, radius, vertebrae and hip) was increased showing a skeletal improvement in treated dtd mice. This improvement was also confirmed by a significant increase of body weight (6.22 g vs 5.08 g; $P < 0.01$) and length (49.17 mm vs 44.23 mm; $P < 0.001$) in dtd treated mice compared to dtd untreated ones. Histology of the growth plate showed an amelioration of its architecture in NAC treated dtd mice compared with untreated animals, further suggesting correction of the endochondral ossification process. The improvement of the bone phenotype of NAC treated dtd mice compared with untreated ones was demonstrated by an increase of femur and tibia BMC (3.56 vs 1.25 mg; $P < 0.001$).

Conclusion(s): Overall, our results demonstrated that NAC is an alternative source of intracellular sulfate through its catabolism paving the way for a pharmacological treatment of diastrophic dysplasia patients.

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P203

Changes in ECM components in Osteogenesis Imperfecta type V

William Lam, Ka-Wai Wong, Danny Chan, Kai-Tsun Michael To
The University of Hong Kong, Faculty of Medicine, Hong Kong, Hong Kong

Background/Introduction: Osteogenesis Imperfecta type V (OI-V) is rare genetic bone disease caused by an autosomal dominant mutation at the 5'UTR of IFITM5 resulting in an addition of 5 amino acids at the N-terminus of the gene product. Clinically, OI-V patients present various degrees of bone fragility and deformity, and with or without characteristic heterotrophic bone growth and hyperplastic callus formation. However, in line with current reports, our clinical specimens near fracture sites show a consistent presence of mesh-like bone lamella, especially around Haversian canals, and increased lacuna density, suggesting problems in extracellular matrix (ECM) organization and/or osteoblast and osteocyte functions.

Purpose: Since mesh-like lamella is consistent and, based on other reports, specific to type V OI, to understand more on the disease mechanism, we decided to investigate the differences in ECM and cellular proteins produced by osteoblast-like cells cultured from three OI-V patients' and three non-OI individuals' bone samples, and validated some of the results with immuno-staining of clinical specimens.

Methods: Osteoblast-like cells were cultured in osteogenic condition, and samples were collected at regular time points for Alizarin Red staining and protein isolation followed by mass spectrometry. Newly synthesized proteins were labeled with heavy isotopes for 24 hours using SILAC system.

Results: OI-V cells show a significantly lower H/L ratio in majority of detected proteins compared with non-OI cells, including most of the

matrisome. Specifically, proteins involved in cytoskeleton and cell-matrix interaction, such as VCL, TPM family and integrin subunits etc., are also reduced. Reversely, some of the proteins involved cellular stress show increased H/L ration during osteogenic induction and this is also observed in the immuno-staining of the clinical specimens.

Conclusion(s): Overall, our data provides a more detailed understanding of the ECM components produced by OI-V bone cells in vitro, and some of them can reflects disease situation in patients.

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New Data Abstracts

P204 (ND)

Osteoblast cell migration during zebrafish fin regeneration

Ivonne Sehring, Gilbert Weidinger

Ulm University, Institute of Biochemistry and Molecular Biology, Ulm, Germany

Background/Introduction: Zebrafish have a high capacity for regeneration and can [GW1] restore lost appendages. Bone regeneration in the zebrafish caudal fin is, at least in part, achieved by the plasticity of mature, differentiated osteoblasts which line the bony elements of the fin, the rays. Amputation induces osteoblasts close to the injury site to dedifferentiate to bone progenitors, which migrate towards the amputation plane.

Purpose: The aim of this study is to identify potential changes in osteoblast morphology, the role of the actin cytoskeleton, and potential signal cues for guided migration during bone regeneration.

Methods: Transgenic fish expressing *bglap*:GFP in mature osteoblasts were imaged at 0 and 1 days post amputation (dpa) for quantification of GFP+ osteoblast migration *in vivo* ($n = 44$-58 rays). 1 dpa fins were stained for the pan-osteoblastic marker Zns5 for cell shape quantification ($n = 5$-10 rays). For pharmacological studies, compounds were injected intraperitoneally.

Results: Osteoblasts close to the amputation plane drastically change their morphology. Their shape changes from roundish to elongated with long protrusions, and they re-orientate and align along the proximo-distal axis. Pharmacological interference with actomyosin dynamics (cytochalasin B 30 μM, blebbistatin 750 nM) impaired osteoblast elongation ($p < 0.0001$) and re-orientation ($p < 0.0001$), and also reduced osteoblast migration ($p < 0.0001$). Inhibition of the complement system, by either inhibiting the receptor for complement component 5a C5aR (W54011 10 μM, PMX-205 10 μM) or the receptor for component 3 C3R (SB290157 10 μM), impaired both osteoblast elongation ($p < 0.0001$) and re-orientation ($p < 0.0001$), and migration towards the amputation plane ($p < 0.0001$).

Conclusion(s): Our data indicate that a dynamic actomyosin cytoskeleton is required for osteoblast plasticity, and suggest that the complement system acts as a guidance for directed osteoblast migration after amputation.

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P205 (ND)

Tissue-level mechanical stimuli drive bone formation and resorption in humans and mice

Matthias Walle[a], Francisco C. Marques[a], Nicholas Ohs[a], Michael Blauth[b], Ralph Müller[a], Caitlyn J. Collins[a]

[a]*ETH Zurich, Department of Health Sciences and Technology, Zürich, Switzerland*

[b]*Innsbruck University Hospital, Department for Trauma Surgery, Innsbruck, Austria*

Background/Introduction: Animal studies have demonstrated a tempo-spatial link between tissue loading and bone remodelling, i.e. mechanoregulation. Although humans and mice share many similarities, evidence for clinical translation of this microscale-mediated relationship is still insufficient.

Purpose: This study aimed to quantify and compare the portion of bone remodelling attributed to mechanics *in vivo* in humans and mice.

Methods: Micro-CT scans (10.5 μm) of eight mice caudal vertebra (CV), cyclically loaded with 8N or 0N, and distal radius (DR) HR-pQCT scans (60.7μm) of seven patients were obtained from previous studies. Bone formation, quiescence, and resorption sites were derived from two subsequent registered scans. Physiological strain energy density (SED) was estimated. Conditional probabilities (CP) were calculated associating surface remodelling events with SED levels. To quantify the proportion of mechanoregulated remodelling, formation (T_f) and resorption (T_r) strain thresholds were varied until a maximum correct classification rate (CCR) was found.

Results: Bone was most likely to be formed in high, quiescent in medium, and resorbed in low SED regions (**Figure 1**). CCR was 0.42 for CV, and DR. Formation occurred above equivalent T_f thresholds ($27.5 \pm 4.89\%$); yet, resorption occurred at higher T_r for CV ($10.5 \pm 1.36\%$) than DR ($2 \pm 0.29\%$). Loading increased CCR ($p<0.05$, CCR=0.43) compared to controls (CCR=0.40) but had no significant effect on remodelling thresholds.

Conclusion(s): This indicates that an increasing stimulus may increase the proportion of mechanoregulated bone remodelling without affecting cell mechanosensitivity. Overall, these results suggest that human and mouse bone remodelling reacts similarly to day-to-day mechanical loads, yet bone resorption thresholds may be more strictly controlled in humans.

2352-1872/$ – see front matter

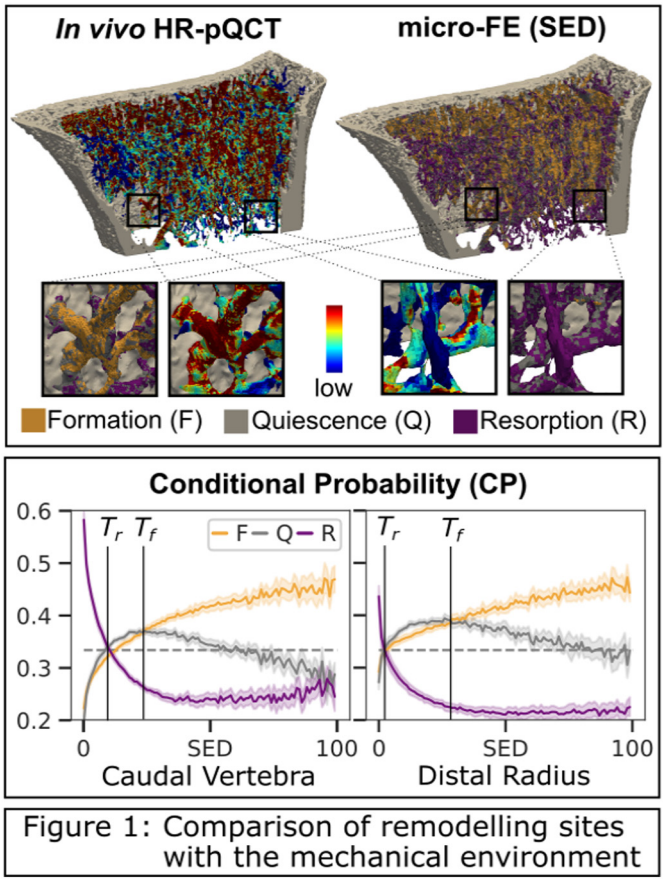

Figure 1: Comparison of remodelling sites with the mechanical environment

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P206 (ND)

Identification of skeletal deformities towards deep phenotyping of zebrafish (Danio rerio) connective tissue disease models

Caitlin Debaene, Adelbert De Clercq, Andy Willaert, Paul Coucke
Ghent University, Biomolecular Medicine, Ghent, Belgium

Background/Introduction: Skeletal deformities in teleost fish have already been extensively described in studies on Atlantic salmon and zebrafish. Nevertheless, a toolset encompassing thorough identification and description of vertebral column deformities to study zebrafish models for human diseases with skeletal involvement is currently lacking.

Purpose: A detailed characterization of skeletal deformities by identifying, describing and quantifying the anomalies will facilitate the development of a reliable deep-phenotyping tool. This tool can be used to establish data matrices by scoring anomalies present in different zebrafish models, which can be used to quantitatively distinguish mild and severe phenotypes. The ability to determine phenotypic severity in disease models is extremely valuable for proper translation towards human diseases, but also to reveal candidate modifier genes that contribute to intrafamilial skeletal variability.

Methods: Zebrafish, 13 *col1a1a*$^{mh13/+}$, 13 *col1a1a*$^{dc124/+}$, 11 *col1a2*$^{mh15/+}$ and 27 WT siblings, were fixed, and made translucent with a mixture of 4% formalin, Triton X-100 and potassium hydroxide (KOH). Subsequently, whole mount bone staining was performed with an Alizarin red S/KOH solution, followed by clearing in a glycerol series. Observations of the skeleton were made using a binocular microscope (Leica M165FC) with a fluorescent unit and equipped with a Leica DFC 450 C camera.

Results: In total, 15 skeletal deformity types were identified and defined: (i) fusion, (ii) compression, (iii) vertical shift of the vertebra, (iv) fractures, (v) curvy ribs), (vi) extra intramembranous bone on the arches and spines (associated elements)and vertebral centra, (vii) bent associated elements, (viii) double associated elements, (ix) detached associated elements, (x) notochord tissue mineralization, (xi) intervertebral ligament mineralization, (xii) lordosis, (xii) kyphosis, (xiii) scoliosis and (xiv) torsion of the vertebral column around the central axis.

Conclusion(s): Deep phenotyping of zebrafish models for skeletal disease will lead to better understanding of expressed phenotypes and of the underlying mechanisms and may lead to identifying new therapeutic targets.

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P207 (ND)

Another player in the game: BMP signaling directly activates chordoblasts for notochord sheath mineralization and centra growth in zebrafish

Hans-Martin Pogoda, Iris Riedl-Quinertz, Matthias Hammerschmidt
Universität zu Köln, Biology/Zoology/Developmental Biology, Köln, Germany

Background/Introduction: Segmented domains within the notochord epithelium express Ectonucleoside Triphosphate Diphosphohydrolase 5a (ENTPD5a) to allow for an iterative mineralization of the collagenous notochord sheath and thus chorda-centra formation, in zebrafish larvae. Thus, the segmented anlage of the developing spine is laid out. Although Notch and Retinoic acid (RA) signaling are already known to feed into this process, the overall molecular machinery controlling the precisely patterned notochord sheath-ossification remains far from understood.

Purpose: Identification of additional molecular players with crucial functions in early zebrafish spine formation.

Methods: We used transgenic approaches to either increase or abrogate BMP activity, both globally and specifically in chordoblasts, the cell type that constitutes the notochord epithelium. We further applied transgene-mediated ablation of chordoblasts, as well as RA treatments. Phenotypes were assessed by reporter-transgene expression, TEM, immunohiostochemistry, and by *in-vivo* of labeling of mineralized matrix.

Results: We found BMP signaling to be sufficient and required for regulation of *entpd5a* activity within the chordoblast layer, subsequently causing block-centra formation or complete loss of mineralization along the notochord, respectively. Furthermore, sustained abrogation of BMP activity after centra induction leads to decreased growth of these structures.

Via immunohistochemistry detecting the intracellular BMP-signal transducer pSmad1/5/8, chordoblast ablation in parallel to BMP2b overexpression, and chordoblast-specific activation or inhibition of BMP signaling, we identified the notochord epithelium cells as the direct targets of BMP signaling. We also found that in the absence of BMP activity, RA is unable to induce the previously described hyper-ossification along the notochord.

Conclusion(s): We identified BMP signaling as another crucial and direct regulator of *entpd5a* activity within the chordoblast layer. Furthermore, it appears to act epistatically to RA in this context.

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P208 (ND)

Adipocyte accumulation-induced increased tumor burden can be abrogated by antagonization of PPARγ

Anastasia Gaculenko[a], Gasper Gregoric[b], Zhengquan Wu[a], Lorenz C. Hofbauer[c], Georg Schett[a], Tobias Bäuerle[b], Aline Bozec[a]
[a]*Universitätsklinikum Erlangen, Department of Medicine 3, Erlangen, Germany*
[b]*Universitätsklinikum Erlangen, Radiological Institute- Preclinical Imaging Platform Erlangen, Erlangen, Germany*
[c]*Technische Universität Dresden, Department of Medicine III and University Center for Healthy Aging, Dresden, Germany*

Background/Introduction: Obesity has been linked to cancer progression in primary tumors in the past decade, though the relationship to secondary tumor manifestations remains unclear. This is especially true for metastasis formation with skeletal involvement, which is to date an abundant cause of death in tumor patients.

Purpose: In our work, we aimed to elaborate whether accumulation of adipocytes in the bone marrow causes the bone niche to be preferable for tumor cell infiltration and if so, why, and how to treat it.

Methods: Obesity in mice, accompanied by a strong increase in adipocyte accumulation in the bone marrow, was achieved by implementing a high fat diet (HFD) into the tumor models. By utilizing imaging techniques (MRI, CT), histology (IF, histomorphometry/-chemistry) and molecular analysis (qPCR, proteome) in two murine species (mice, rats) and two metastasis setups (melanoma, breast cancer), we covered a broad spectrum of experimental approaches. The animals were subjected to tumor cell inoculation methods inducing bone metastasis.

Results: Both murine species showed increased soft tissue and osteolytic tumor burden when being obese. IF of human bone metastasis biopsies confirmed our previous observations by showing a correlation (R^2=0.6372) between proliferation and adipocyte signal. As treatment approach, we applied the PPARγ antagonist Bisphenol-A-diglycidylether (BADGE) as adipocyte differentiation and storage inhibitor to lean and obese animals. Obesity-induced increase in osteolytic tumor burden was significantly (p=0.006) decreased with treatment. This development was accompanied by a decrease in pro-tumorigenic, inflammatory, and osteoclastogenic markers as well as a normalization of bone remodeling parameters, indicating increasing bone strength.

Conclusion(s): In conclusion, our study could show that adipocyte accumulation induces an increase in bone tumor burden, which can be reversed by inhibiting PPARγ. This opens up a novel treatment approach in obese patients to prevent loss in bone strength and prolong survival.

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P209 (ND)

Involvement of FXR in the OPG/RANKL pathway of breast and prostate cancer cells

Lara Absil[a], Emilie Rigaux[a], Lionel Tafforeau[b], Jean-Jacques Body[c], Denis Nonclercq[a], Fabrice Journe[d]
[a]*University of Mons, Histology, Mons, Belgium*
[b]*University of Mons, Cell Biology, Mons, Belgium*
[c]*Brugmann University Hospital, Medicine, Brussels, Belgium*
[d]*University of Mons, Human Anatomy and Experimental Oncology, Mons, Belgium*

Background/Introduction: Bone is the first and most common distant metastatic site for breast and prostate cancers. Such metastases complicate cancer management, induce considerable morbidities and decrease patient quality of life and survival. Osteomimetism is part of the complex process of osteotropism of cancer cells. Our recent clinical and experimental data highly support a relationship between the expression of the bile acid receptor FXR in breast cancer and the propensity of tumor cells to develop bone metastases. RUNX2 is involved in this process and, subsequently, promotes synthesis of bone proteins, such as osteopontin, osteocalcin and bone sialoprotein (Absil et al., BMC Cancer, 2020).

Purpose: In the present study, we examined the impact of cancer cells on the OPG-RANK-RANKL pathway by assessing RANKL and OPG expression in breast and prostate cancer cell lines and FXR involvement in their regulation.

Methods: OPG and RANKL protein expression levels were evaluated by immunofluorescence in breast (MCF7, MDA-MB-231) and prostate (LNCap, PC3) cancer cell lines exposed to FXR agonist (chenodeoxycholic acid, CDCA) and/or antagonist (lithocholic acid, LCA). FXR depletion was conducted using siRNA strategy.

Results: We showed that FXR activation by the CDCA agonist significantly increased OPG expression in breast (130%) and prostate (115%) cancer cells ($p<0.05$, Anova and post hoc Dunnet's test), but did not change RANKL levels. Moreover, FXR inhibitors used in combination with CDCA decreased OPG expression and had no effect on RANKL. Silencing RNA against FXR validated OPG and RANKL results in breast cancer cells.

Conclusion(s): Therefore, FXR in metastatic cells may play a role (i) in the balance between bone formation and resorption, and (ii) probably in the survival of cancer cells by stimulating the production of OPG, a decoy receptor for RANK and TRAIL pathways.

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P210 (ND)

Whole-Body Vibration (WBV) affects perichordal and endochondral bone development and patterning in zebrafish larvae

Shirine Jeradi, Tamara A. Franz-Odendaal
Mount Saint Vincent University, Biology, Halifax, Canada

Background/Introduction: Whole body vibrations (WBV) are a potentially harmful non-chemical pollutant that can have severe effects on developing embryos. This is especially relevant at early stages of embryonic development, when organisms are most susceptible to defects and abnormalities.

Purpose: In this study, we used zebrafish embryos and larvae, aged between 10 hours post-fertilization hpf and 5 days post fertilization (dpf) to decipher the effect of exposure to WBV on the bone tissue of a living organism, during bone development and maintenance.

Methods: A vibration platform was developed to expose zebrafish embryos and larvae to controlled levels of WBV. Zebrafish aged between 10hpf and 5dpf were exposed to WBV frequencies of 20Hz (Low frequency vibrations) for up to 4 days. Fish were either fixed immediately after WBV treatment and used to perform *In Situ Hybridization* ISH or raised to SL5.5-6.6mm prior to fixation to perform Alcian Blue/Alizarin Red staining. These experiments were approved by the institutional Animal Care Committee, in line with the CCAC guidelines.

Results: Despite their mildness, these treatments were sufficient to induce a wide array of skeletal defects in zebrafish larvae. Depending on the developmental stage at which the exposure to WBV was started, different skeletal elements of zebrafish larvae were affected. WBV also differentially affected perichordal and endochondral bones. In fact, fish exposed to WBV starting at 10hpf were missing their ural (perichordal) bones, while exposure to WBV starting at 4 and 5 dpf primarily affected the hypural (endochondral) bone. ISH revealed the WBV effect on the hypural bone development was independent of *sox9a* expression.

Conclusion(s): These results indicate that exposure to WBV during embryonic development can affect the normal skeletal development in vertebrates. Translating our findings to human embryonic development can substantially contribute to improve workplace safety practices for workers utilizing industrial machinery in many sectors.

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P211 (ND)

Frequency and amplitude analyses of calcium oscillations reveals the harmony regulated by ITAM receptors during RANKL-induced osteoclastogenesis

Hiroyuki Okada[a], Hiroshi Kajiya[b], Yasunori Omata[c], Hironori Hojo[d], Ung-il Chung[d], Koji Okabe[b], Takeshi Miyamoto[e], Sakae Tanaka[c]
[a]*the University of Tokyo, Center of Disease Biology and Integrative Medicine- Department of Orthopaedic Surgery, Tokyo, Japan*
[b]*Fukuoka Dental College, Department of Physiological Science and Molecular Biology, Fukuoka, Japan*
[c]*the University of Tokyo, Department of Orthopaedic Surgery, Tokyo, Japan*
[d]*the University of Tokyo, Center of Disease Biology and Integrative Medicine, Tokyo, Japan*
[e]*Kumamoto University, Department of Orthopaedic Surgery- Faculty of Life Science, Kumamoto, Japan*

Background/Introduction: RANKL-induced Calcium oscillations mediated by ITAM costimulatory receptors are considered essential in osteoclastogenesis. However, contribution of FcRgamma and DAP12 to oscillations in differentiational time-course remains unknown. In addition, whether M-CSF or RANKL evokes oscillations is still unclear.

Purpose: To clarify the different role of each ITAM receptor in early osteoclastogenesis with a novel analytical method for the spectrum and the amplitude of oscillations.

Methods: Bone marrow macrophages (BMMs) after 2-day M-CSF treatment in four phenotypes of mice were subjected to experiments; WT, FcRgamma, DAP12, and ITAMs KOs. Some BMMs were treated with M-CSF or RANKL for additional two days. After stained with a calcium indicator fura2, ratio of fluorescence emission in each cell was recorded with fluorescence microscope every 2 to 3 seconds. Platform R and Stan were used for analyses.

Results: Spontaneous oscillations without RANKL treatment could be detected from all phenotypes mice even in ITAMs DKO mice. Frequency analyses showed RANKL-treated BMMs from DAP12 KO mice consisted of more low frequency waves. Taking individual difference and observational errors into consideration, state space modeling revealed the amplitude of oscillations were elevated both by M-CSF and by RANKL. The amplitude of oscillations from DAP12 KO BMMs were elevated before cytokines' stimulation, in contrast, suppressed by RANKL treatment.

Conclusion(s): Our novel frequency and amplitude analyses of calcium oscillations showed DAP12 suppressed low frequency range and maintained the amplitude after RANKL stimulation. DAP12 is a key controller of oscillations in RANKL-induced osteoclastogenesis.

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P212 (ND)

Insights into bisphosphonate mediated jaw necrosis from a zebrafish model

Lalith Prabha Ethiraj, Tom James Carney
Nanyang Technological University, Lee Kong Chain School of Medicine, Singapore, Singapore Rep. of

Background/Introduction: Through their osteoclast inhibitory activity, bisphosphonates are used to treat a variety of bone disorders including osteoporosis, osteogenesis imperfecta (OI), metastatic bone disease and hypercalcemia of malignancy. Their use has expanded in recent years, however this has led to a concomitant increase in the incidence of Bisphosphonate related Osteonecrosis of Jaw (BRONJ), a rare but serious side effect of bisphosphonates. The rarity of BRONJ limits the power of human studies, and the underlying mechanism remains unclear.

Purpose: The aim of this present study is to show zebrafish pharyngeal teeth are a site of high spontaneous osteoclastic activity. Pharmacological suppression of osteoclast affects pharyngeal bone, leading to BRONJ type of lesions.

Methods: We have recently demonstrated efficacy of alendronate in zebrafish OI and fracture models. In this study, quantification of osteoclastic activity on dissected zebrafish pharyngeal teeth, following 24h exposure to a range of alendronate concentrations, is done.

Results: We now show that alendronate treatment causes reduction of osteoclast activity, both in bone fractures and in tooth bearing ceratobranchial arches. In the latter, alendronate treatment also robustly leads to pharyngeal teeth and bone disjunction, causing mobility of teeth as a mild phenotype and shedding of teeth as severe phenotypic changes. This exfoliation of teeth resembles loss of teeth observed in BRONJ patients, thus highlighting zebrafish as a valid BRONJ model. Severity of tooth exfoliation is proportional to concentration and treatment duration, $n=10$ per group. **** $p<0.0001$, ANOVA with Brown-Forsythe test.

Conclusion(s): These findings demonstrate that alendronate treatment in zebrafish suppresses osteoclastic activity in the jaw, and also yields teeth-bone disjunction, as observed clinically.

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P213 (ND)

Nutritional characteristics and body composition of patients undertaken for hip and knee arthroplasty

María Martín Fuentes[a], Blanca Varas de Dios[b]
[a]*Santa Cristina Universitary Hospital, Endocrinology, Madrid, Spain*
[b]*Santa Cristina Universitary Hospital, Rheumatology, Madrid, Spain*

Background/Introduction: Malnutrition, both due to deficiency and excess of nutrients, is related to the morbidity of the surgical patient.

Purpose: Evaluate the nutritional status and body composition in patients who are going to undergo elective knee and hip arthroplasty.

Methods: An observational cross-sectional study is carried out evaluating patients who are admitted on a scheduled basis for hip and knee replacement surgery from February to September 2019 at the Santa Cristina Hospital. Upon admission, nutritional screening is performed using the Malnutrition Universal Screening Tool (MUST), anthropometric measurements, manual dynamometry, analytical with nutritional parameters and assessment of body composition with bioimpedanciometry (BIA101 Akern).

Results: 86 patients (61.6% women) were evaluated, mean age 69.5 ± 9.5 years. 55.8% were knee surgeries. According to MUST, 21.3% were at risk of malnutrition. 59% had obesity (mean BMI 31.3 ± 4.5). In anthropometry, 6.8% had a decreased arm muscle circumference, 12.2% had a calf circumference, and 16.9% had a triceps crease with respect to the 50th percentile. 88.7% had a pathological waist measurement. 20% had manual dynamometry below reference values. In 91.4%, vitamin D <30 pg / ml, 19% prealbumin ≤ 18.

Conclusion(s): In the assessment of body composition, women had significantly lower total muscle mass, appendix, lower% of total body

water and a greater amount of fat mass, compared to men, also presenting decreased parameters compared to those of reference.

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P214 (ND)

Search for epigenetic markers of osteoporosis formation

Bulat Yalaev, Rita Khusainova
Institute of Biochemistry and Genetics - Subdivision of the Ufa Federal Research Centre of the Russian Academy of Sciences, Laboratory of Human Molecular Genetics, Ufa, Russia C.I.S.

Background/Introduction: There are data on the possible role of polymorphic variants of microRNA target sites in genes of bone metabolism in the development of osteoporosis (OP). However, research findings are inconsistent across populations.

Purpose: The objective of our study was to search for associations of rs11540149, rs6854081, rs10098470, rs10793442, rs1054204, rs1061947, rs1042673, rs9659030, rs1031820, rs5854, rs198470, rs1712, rs2745426 in microRNA target sites and rs2910164, rs11614913 in microRNA genes in postmenopausal women and men over 45 years of age with a low level of BMD and the risk of osteoporotic fractures.

Methods: DNA samples of 663 postmenopausal women and 508 men over 45 years old from the Volga-Ural region of Russia were studied. Genotyping was carried out by competitive allele-specific PCR KASP™. The search for associations of polymorphic variants with OP endophenotypes was carried out using the Pearson $\chi 2$ criterion.

Results: There was a significant association of Allele A of polymorphic variant rs11540149 with fractures in general ($p=0.043$) and spinal fractures ($p=0.016$), allele G rs6854081 with hip fractures ($p=0.00632$) and fractures in general ($p = 0.042$), C allele rs2910164 with low BMD in the lumbar spine ($p=0.000102$), T allele rs10098470 with fractures in general ($p=0.039$), allele A rs10793442 with low BMD in the general sample ($p=0.041$). In men: A allele rs11540149 is associated with fractures ($p=0.041$) and low BMD in the general sample ($p=0.002$), as well as with fractures of the radius ($p=0.00374$), T allele rs11614913 with fractures in general ($p=0.03275$), G allele rs6854081 with femoral neck fractures ($p=0.02469$), GG genotype at locus rs1054204 with low BMD in the general sample ($p=0.03$).

Conclusion(s): The association of polymorphic variants of miRNA target site with a low level of BMD and the risk of fractures in genes that are involved in the regulation of bone metabolism was revealed.

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P215 (ND)

Sympathectomy as well as β2-adrenoceptor deficiency lead to exacerbation of subchondral bone changes in experimental osteoarthritis

Gundula Rösch[a], Dominique Muschter[b], Karima El Bagdadi[a], Shahed Taheri[c], Andrea Meurer[a], Arndt F. Schilling[c], Susanne Grässel[b], Rainer H. Straub[d], Frank Zaucke[a], Zsuzsa Jenei-Lanzl[a]
[a]*Dr. Rolf M. Schwiete Research Unit for Osteoarthritis- Department of Orthopedics Friedrichsheim, University Hospital Frankfurt- Goethe University, Frankfurt, Germany*
[b]*Department of Orthopedic Surgery- Exp. Orthopedics, ZMB/Biopark 1- University of Regensburg, Regensburg, Germany*
[c]*Department of Trauma Surgery- Orthopedics and Plastic Surgery, University Medical Center Goettingen, Goettingen, Germany*
[d]*Laboratory of Experimental Rheumatology and Neuroendocrine Immunology, Department of Internal Medicine- University Hospital Regensburg, Regensburg, Germany*

Background/Introduction: Recent *in vitro* studies demonstrated that the sympathetic nervous system (SNS) and its major neurotransmitter norepinephrine might contribute to OA progression mediated by α1/2 or β2-adrenoceptors (ARs). Several AR subtypes are expressed in all joint tissues.

Purpose: To unravel their role during OA pathogenesis *in vivo we* examined the progression of surgically-induced OA in sympathectomized (Syx) and β2-AR-deficient ($Adrb2^{-/-}$) mice.

Methods: OA was induced by destabilization of the medial meniscus (DMM) in wildtype (WT), Syx and $Adrb2^{-/-}$ mice. 8 weeks after DMM or sham surgery, subchondral bone was analyzed by μCT and the severity of OA by histological scoring.

Results: Bone volume fraction (BV/TV, Fig.1) (WT 0.787 ± 0.021, Syx 0.885 ± 0.025, $Adrb2^{-/-}$ 0.942 ± 0.025), trabecular thickness (WT 0.249 ± 0.033 μm, Syx 0.323 ± 0.043 μm, $Adrb2^{-/-}$ 0.506 ± 0.085 μm) and subchondral bone plate thickness (WT 107.7 ± 3.1 μm, Syx 128.5 ± 3.5 μm, $Adrb2^{-/-}$ 160.0 ± 14.3 μm) were significantly increased in Syx and $Ardb2^{-/-}$ compared to WT mice after DMM, while there were no significant differences between WT, Syx and $Adrb2^{-/-}$ animals after sham surgery. The progression in cartilage degeneration and synovial inflammation was comparable in WT, Syx and $Ardb2^{-/-}$ DMM mice without significant differences.

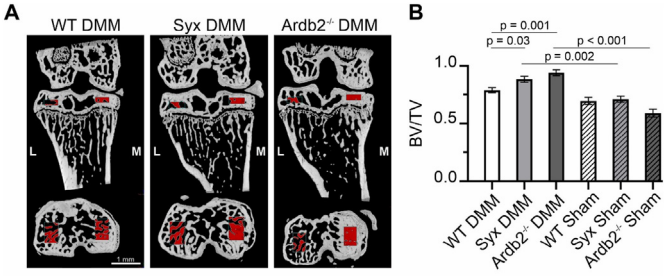

Fig.1: μCT analysis of subchondral bone microarchitecture (A) representing the volume of interest (M = medial, L = lateral) and (B) the analysis for BV/TV.

Conclusion(s): The increased bone mass in Syx and Adrb2-/- DMM mice suggests that there are synergistic effects of the SNS and OA in subchondral bone, mainly through β2-AR deficiency. Taken together, the β2-AR plays a major role in OA-related subchondral bone changes and is therefore an attractive target for novel therapeutic avenues.

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P216 (ND)

Cripto favours chondrocyte hypertrophy via TGF-beta SMAD1/5 signaling in experimental osteoarthritis

Gonzalo Sanchez-Duffhues[a], Amaya Garcia de Vinuesa Antuñano[a], Esmeralda Blaney-Davidson[b], Arjan van Caam[b], Kirsten Lodder[a], Yolande Ramos[c], Margreet Kloppenburg[c], Ingrid Meulenbelt[c], Peter van der Kraan[b], Marie-José Goumans[a], Peter ten Dijke[a]
[a]*Leiden University Medical Center, Cell and Chemical Biology, Leiden, Netherlands*
[b]*Radboud University- Nijmegen Medical Centre, Experimental Rheumatology & Advanced Therapeutics, Nijmegen, Netherlands*
[c]*Leiden University Medical Center, Department of Biomedical Data Sciences, Leiden, Netherlands*

Background/Introduction: Osteoarthritis (OA) is a painful and disabling condition of the joints affecting millions of people, for which effective biomarkers and therapies are desperately required. OA

chondrocytes exhibit an aberrant response to the secreted cytokine Transforming Growth Factor (TGF)-β, enhancing intracellular signaling downstream of the type I kinase receptor kinase ALK1 at the expense of ALK5 mediated signaling. Unfortunately, the underlying mechanisms remain elusive.

Purpose: To identify druggable targets for OA investigating molecules regulating the ALK1/ALK5 balance in OA chondrocytes.

Methods: Gene expression of TGF-β signaling modulators was analyzed in joints from three different mouse models of OA, and protein expression validated in murine and human cartilage OA samples. *In vitro* differentiation and biochemistry assays in ATDC5 mouse chondrocyte and *ex-vivo* mouse metatarsal assays were used to investigate functional alterations in TGF-β signaling.

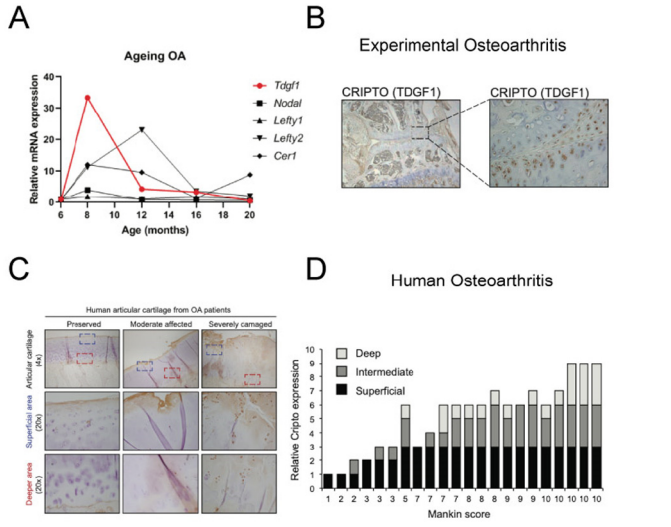

Figure 1. Cripto expression correlates with OA development. A) Gene expression analysis during age-induced osteoarthritis in C57BL/6N mice. RNA was isolated from cartilage of C57BL6 mice at different ages (6, 8, 12, 16, 20 months old). Relative mRNA expression of Tdgf1 (encoding Cripto), Nodal, Lefty1, Lefty2 and Cerberus (Cer1) compared to 6 months old is shown. (RNA pooled from 8-11 mice per group). B) Immunohistochemical analysis of Cripto expression in paraffin sections from 8 months old C57BL/6N mice joints (magnification 10X and 40X in zoomed image). C) Immunohistochemical analysis of Cripto expression on paraffin slides from OA patients. Superficial and deeper layers of cartilage samples are shown, comparing different degrees of damage within the same section. Representative sections are shown (Magnification 10X and 20X for zoomed images). D) Correlation of Cripto expression and Mankin score (for OA staging) in human OA samples.

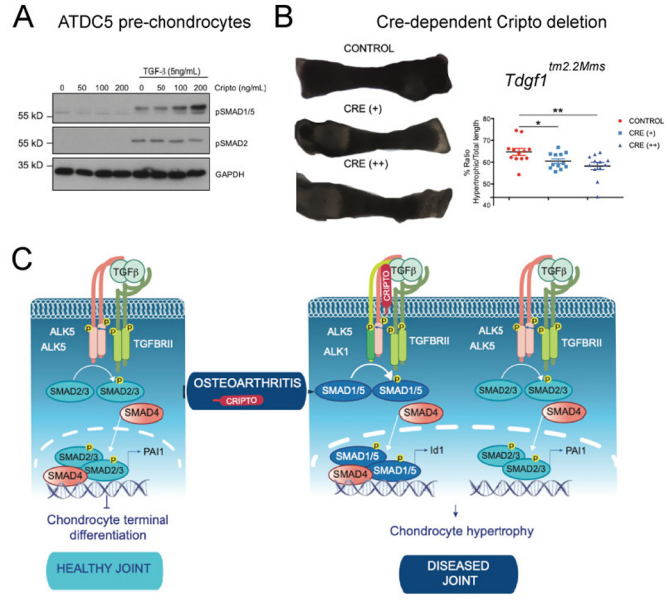

Figure 2. Cripto modulates TGF-β-Smad1/5 signaling and chondrocyte hypertrophy. A) Western blot analysis in ATDC5 cells serum starved and preincubated for 30 mins with recombinant Cripto and stimulated with TGF-β for 45 mins. B) Representative bright field images of E17.5 Tdgf1tm2.2Mms metatarsals incubated for 10 days with control lentiviruses or increasing concentrations of Lentiviral particles encoding the Cre recombinase (magnification 4X) and corresponding quantification analysis (n=12). C) Graphical abstract.

Results: Cripto (*Tdgf1*) is increased in murine experimentally induced OA and human OA chondrocytes. Mechanistically, Cripto participates in a TGF-β-ALK1-Cripto receptor complex in the plasma membrane, thereby inducing catabolic SMAD1/5 signaling. Cripto over-expression favors the hypertrophic differentiation of chondrocytes, eventually contributing to tissue calcification.

Conclusion(s): The TGF-β coreceptor Cripto is expressed in OA and plays a functional role promoting chondrocyte hypertrophy, thereby becoming a novel potential biomarker and/or therapeutic target in OA.

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P217 (ND)

Septic knee arthritis in the emergency department of a tertiary hospital

Blanca Varas de Dios
Santa Cristina Universitary Hospital, Rheumatology, Madrid, Spain

Background/Introduction: Septic arthritis (SA) of the knee is a medical emergency that requires prompt attention and treatment in order to avoid permanent damage to the joint. There´s no consensus on emergency management in Spain.

Purpose: To review the behavior in the emergency room in patients diagnosed with SA and to compare it with a management guide validated in another country.

Methods: Descriptive, retrospective study of positive fluids from native joints. Data were obtained in the first hospital contact and evolution. The diagnostic and therapeutic approach was compared with the Guide for the management of the swollen and warm joint published by the British Society of Rheumatology.

Results: 46 SA cases were included after being admitted from the Emergency Department. Mean age was 62.3±6.3 years (21 to 89), 54.3% male. Background: DM 4 cases (8.6%), diabetic neuropathy 1 (2.1%), previous AS 5 (10.8%), history of skin opening 8 (17.3%), infection of skin without opening 7 (15.2%) and previous arthrocentesis 6 (13.0%). The determination of CRP and ESR with clinical suspicion was performed in 10 (21.7%) and 14 (30.4%) cases. Blood cultures performed in 29 cases (63.0%). Arthrocentesis recommendations and joint fluid analysis: In 40 (86.9%) of the 46 cases, suspicion of SA was established in the first consultation, with arthrocentesis performed in 37/40 (92.5%). The samples were sent for GRAM study 19 (41.3%), microbiological culture 44 (95.6%), leukocyte count 26 (56.5%) and study of microcrystals 4 (8.6%). Recommendations for treatment and follow-up: 100% of the cases were treated with antibiotics empirically and 84.7% (39) according to the recommendations. The request for two ESR/CRP determinations until hospital discharge was made in 44 cases (95.6%). IV antibiotic treatment time 22.90±6.12 days (7 to 42), oral: 20.08±10.52 (7 to 31) days, total antibiotic therapy time of 35.91±6.75 (21 to 50). Hospitalization: 25.39±6.05 (8 to 50) days. Surgery was necessary in 18 (39%). The time between the first consultation and the start of the empirical antibiotic: 2.1±1.64 (0 to 6) days.

Conclusion(s): This study reviews the cases of SA demonstrated by positive cultures. The diagnostic suspicion must be much higher than the casuistry that we have presented. We admit that it´s on this population that the recommendations of the guidelines should affect. Therefore, some shortcomings are demonstrated regarding the diagnostic management during the first consultation, hence the importance of disseminating the SA management guidelines to reduce the lack of adherence that we have detected.

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P218 (ND)

Computational anatomy and geometric shape analysis enables analysis of complex craniofacial phenotypes in zebrafish mutants

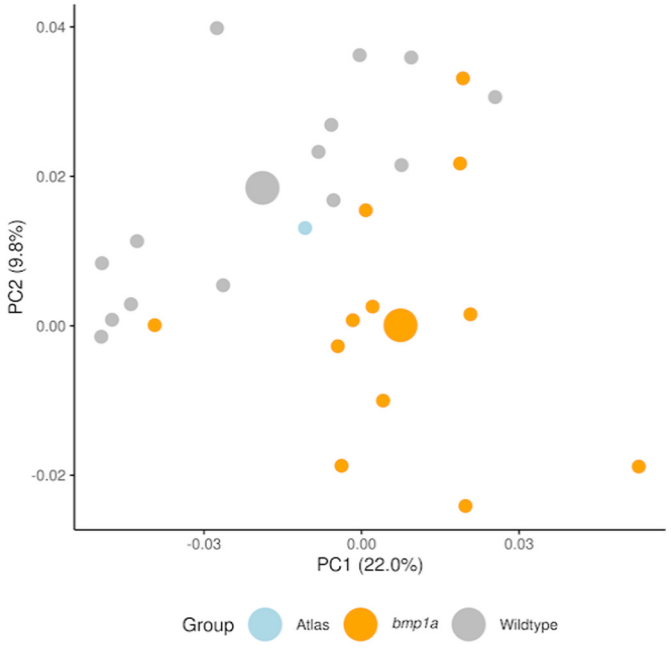
Kelly Diamond[a], Ronald Kwon[b], Murat Maga[a]
[a]*Seattle Children's Research Institute, Center for Developmental Biology and Regenerative Medicine, Seattle, United States*
[b]*University of Washington, Orthopaedics and Sports Medicine, Seattle, United States*

Background/Introduction: Computational anatomy (CA) approaches estimate a 'template' from a sample of images. This template is then used as a basis for statistical analysis to quantify structural differences among groups of interest.

Purpose: In this work we aim to identify and quantify differences in 3D craniofacial phenotypes in adult zebrafish with somatic mutations in *bmp1a*, whose human ortholog *BMP1*, when disrupted, is associated with Osteogenesis Imperfecta.

Methods: We first estimate a synthetic zebrafish template using Advanced Normalization Tools software. To validate the accuracy of the template and our CA pipeline, we compared the otolith volumes derived from the CA approach to manually segmented volumes of the same set of zebrafish. We then used a set of 372 geometrically defined pseudo-landmark points to quantify phenotypic differences between *bmp1a* and wildtype fish using a Generalized Procrustes Analysis (GPA).

Results: Our overall CA based segmentation volumes are statistically similar to our manual segmentations ($t=-0.912$, $p=0.363$) and both results show that mutants have larger otoliths than their wildtype controls. Our GPA separated the *bmp1a* and wildtype fish along the first two principal components which collectively explained 31.8% of the variation in the dataset (Figure 1). Compared to controls, the phenotypic differences in *bmp1a* fish are concentrated around the operculum and the orbit.



Conclusion(s): Our CA approach offers a potential pipeline for high throughput screening of complex fish craniofacial phenotypes, especially those of zebrafish which are an important model system for testing genome to phenome relationships in the study of development and human diseases.

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P219 (ND)

A collagen10a1 mutation disrupts cell polarity and causes skeletal defects in a medaka model for Schmid Metaphyseal Chondrodysplasia

Wen Hui Tan[a], Bich Ngoc Tran[a], Daria Larionova[b], Bertie Joan van Heuven[c], Federica Marone[d], Martin Rücklin[c], Paul Matsudaira[a], Christoph Winkler[a]
[a]*National University of Singapore, Department of Biological Sciences, Singapore, Singapore Rep. of*
[b]*Ghent University, Department of Biology- Research Group Evolutionary Developmental Biology, Ghent, Belgium*
[c]*Naturalis Biodiversity Center, Naturalis Biodiversity Center, Leiden, Netherlands*
[d]*Paul Scherrer Institut, Swiss Light Source, Villigen, Switzerland*

Background/Introduction: Schmid Metaphyseal Chondrodysplasia (SMCD) is an autosomal dominant skeletal disorder caused by mutations in the COL10A1 gene. Affected individuals exhibit irregular growth plates, bowed legs, hip deformities and dwarfism.

Purpose: Analysis of patient samples and SMCD mouse models highlighted a key role for endoplasmic reticulum (ER) stress in the SMCD pathology. However, how an increase in ER stress translates into defects at the bone growth plate remains unclear. To address this question, we generated a medaka fish model for SMCD that is accessible to live imaging at high cellular resolution.

Methods: Using CRISPR/Cas9, a *col10a1*^{633a} medaka mutant that harbors a SMCD-relevant mutation was generated.

Results: Heterozygous *col10a1*^{633a} carriers recapitulated key features of SMCD, such as an increase in ER stress, skeletal deformities and a reduced body length. In addition, γ-tubulin immunostaining and electron microscopy revealed that *col10a1*-expressing cells in *col10a1*^{633a} mutants have atypical microtubule-organizing center (MTOC) localization and a skewed nuclear division axis suggesting impaired cell polarity. In line with this, live confocal microscopy, bone and cartilage stainings, as well as microfocus- and synchrotron-based X-ray tomographic microscopy revealed disorganization of skeletal tissues in mutants.

Conclusion(s): Together, our data highlight the *col10a1* medaka mutant as a novel model for SMCD and imply a critical role for cell polarity defects in SMCD pathogenesis.

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P220 (ND)

Lrp5 gene knockout causes craniofacial deformities and fractures in adult zebrafish

Iryna Khrystoforova[a], Chen Shochat-Carvalho[a], Katherine C. Woronowicz[b,c], Matthew P. Harris[b,c], David Karasik[a]
[a]*Bar-Ilan University-, The Azrieli Faculty of Medicine- David Karasik lab, Safed, Israel*
[b]*Boston Children's Hospital, Department of Orthopaedics, Boston, United States*
[c]*Harvard Medical School, Department of Genetics, Boston, United States*

Background/Introduction: In humans, loss-of-function mutations in Low-density Lipoprotein Receptor-related Protein 5 (*LRP5*) cause Osteoporosis-Pseudoglioma syndrome, a low bone mass disorder, while missense mutations have been observed in individuals with high bone mass. LRP5 is a co-receptor of Wnt-signalling pathway, which controls expression of genes involved in osteogenesis. Like in humans, the zebrafish (*Danio rerio*) skeleton forms either by using cartilage scaffold as a template, or directly (without cartilage scaffold). Genetic determinants that control bone formation are highly conserved between zebrafish and mammals, which was supported by the finding that *lrp5* is required for neural crest cells migration and cranial skeleton morphogenesis of *lrp5* crispants (Willems et al., 2015). However, the systemic effect of lrp5 deficiency and shared functional roles within vertebrates remain unknown.

Purpose: We therefore generated *lrp5* knock-out zebrafish which allowed us to follow skeletogenesis from larval to adult stages in the zebrafish and directly compare its role in regulating skeletal differentiation and development.

Methods: *lrp5* stable knock-out was generated by CRISPR-Cas9 genomic editing. Incrossed carrier progeny was analyzed for mineralized bone matrix and cartilage by Alizarin Red and alcian blue staining across developmental stages. Adult (6 mo) progeny was also scanned by micro-CT Bruker SkyScan 1172 for skeleton phenotype.

Results: Alizarin red staining revealed that notochord mineralization at 7d and 13d of development is delayed and the total mineralization level of 27d and adult's skeleton is lower in *lrp5* knock-out fish. Further, micro-CT scanning of adults demonstrated malformations in the cranial skeleton of *lrp5* mutants, with accumulating fractures observed in parasphenoid and mandible bones. Additionally, we observed decreased whole body bone mineral density in adult mutants.

Conclusion(s): In summary, our mutant analysis demonstrates that lrp5 is important for skeletal differentiation, as its absence results in delayed mineralization throughout the zebrafish axial skeleton at early stages, consequently resulting in deformation of neuro- and viscerocranium.

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P221 (ND)

Variation of shape, bone structure and mineralisation of vertebral centra in young adult chihuahua, a zebrafish model for human classical osteogenesis imperfecta

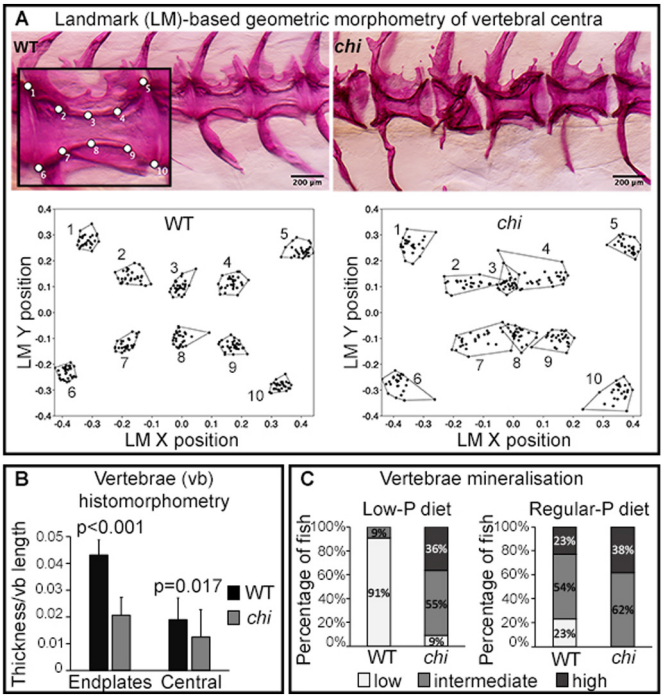
Silvia Cotti[a], Ann Huysseune[a], Antonella Forlino[b], P. Eckhard Witten[a]
[a]*Ghent University, Department of Biology, Gent, Belgium*
[b]*University of Pavia, Department of Molecular Medicine, Pavia, Italy*

Background/Introduction: The *chihuahua* (*chi*) zebrafish is an established model for human classical osteogenesis imperfecta (OI), a bone disorder caused by mutations in collagen type I. Adult *chi* display hallmarks of human OI, i.e. bone deformities, fragility and high mineral content. The *chi* bone phenotype appears at juvenile stages and worsens with age.

Purpose: Analysis of the early *chi* bone phenotype. Given that low dietary phosphorus (P) intake can increase bone matrix formation and prevent bone mineralisation in wildtype zebrafish (Cotti et al. 2020, Int J Mol Sci, 21, 5429; doi:10.3390/ijms21155429), it was tested whether dietary treatment can reduce excess mineral-to-matrix deposition in *chi*.

Methods: One month old wildtype and *chi* zebrafish were fed with low- or regular-P diets for two months (ethical approval 260/2020-PR) and analysed by whole mount bone staining and histological procedures.

Results: Under regular-P diet, *chi* display higher frequencies of kyphosis ($p=0.002$), lordosis ($p=0.005$) and vertebral body compressions ($p<0.001$) compared to wildtype. Landmark-based geometric morphometry reveals a strong shape variation of *chi* vertebral centra compared to wildtype ($p<0.001$) (Fig.1A). In *chi* the thickness of vertebral body bone structures is reduced ($p<0.001$, $p=0.017$) (Fig.1B). Different from wildtype fish, low-P diet does not reduce the mineralisation of *chi* vertebral bodies (Fig.1C).



Conclusion(s): The *chi* vertebral centra are thin, compressed and highly variable in shape. Sustained low levels of bone matrix formation may account for the high level of mineral-to-matrix ratio, even under low dietary P conditions.

Funding: EU-H2020-MSCA-766347, BOFGOA2021000407.

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P222 (ND)

Skeletal variations in wild type medaka: Baseline studies on a biomedical model

Claudia Di Biagio[a], Ann Huysseune[b], P. Eckhard Witten[b], Clara Boglione[a]
[a]*University of Rome- Tor Vergata, Biology, Rome, Italy*
[b]*Gent University, Biology, Gent, Belgium*

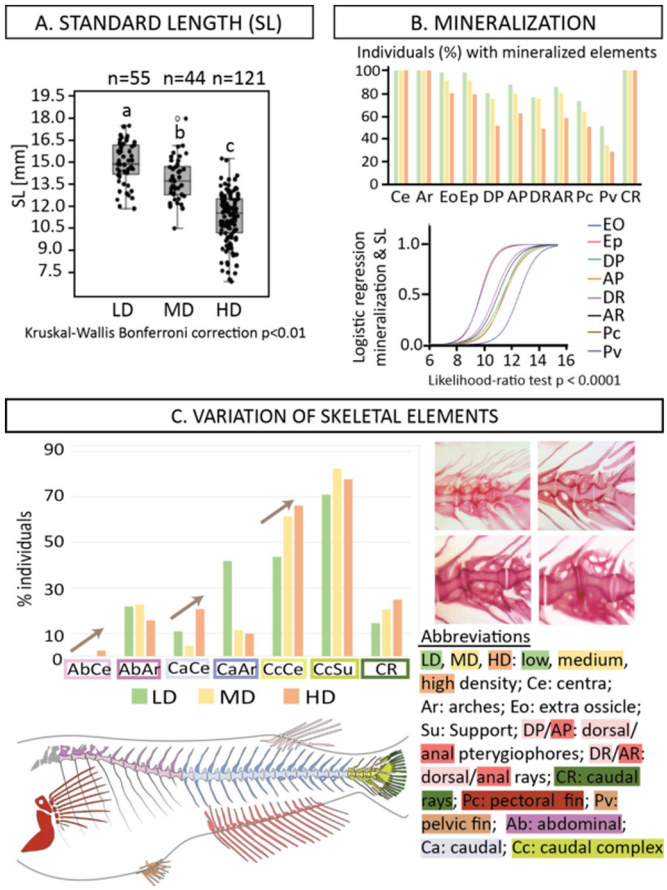
Background/Introduction: *Oryzias latipes* is increasingly used as model for human bone diseases. The variability of skeletal elements under laboratory conditions is an important component of diagnosing mutant phenotypes. Our knowledge about type and extent of skeletal variations in this species is scarce, particularly related to rearing density.

Purpose: Aims are (I) to provide a comprehensive overview of axial skeleton variations in wild type medaka; (II) investigate the

skeletal response to different rearing densities in terms of animal size, number and shape of skeletal elements, mineralisation, and presence of skeletal defects.

Methods: After hatching animals were reared in a recirculating system for 40 days at 3 different densities: low (LD = 5 fishes/L), medium (MD = 15 fishes/L) and high (HD = 45 fishes/L). Ethical approval: 133/2021-PR. The analysis was based on whole mount staining with Alizarin red S, on histological and enzyme histochemical protocols.

Results: HD juveniles had a significantly reduced average length and a wider length distribution than LD or MD animals (Fig.A). A reduced mineralization of skeletal elements in HD animals was correlated with the standard length, indicated by logistic regression analysis (Fig.B). Rearing density had no effect on numbers of vertebral bodies or arches. Vertebral bodies and arches that support the caudal fin are most plastic (Fig.C) concerning shape and fusion of skeletal elements. Vertebral centra and arches are developmental modules, accordingly variations of these elements are uncoupled.



Conclusion(s): A comprehensive axial skeleton overview is provided. Rearing density affects the skeleton of medaka and should be considered when phenotyping. Funding: EU-H2020-MSCA-766347.

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P223 (ND)

Are non-fracture vertebral deformities more prevalent in patients with osteoporotic vertebral fractures?

Margaret Paggiosi[a], Dharshan Giri Neelam Naganathan[b], Uzayr Undre[b], Nicola Peel[c], Richard Eastell[a]
[a]*The University of Sheffield, Oncology and Metabolism, Sheffield, United Kingdom*
[b]*The University of Sheffield, The Medical School, Sheffield, United Kingdom*
[c]*Sheffield Teaching Hospitals NHS Foundation Trust, Metabolic Bone Centre, Sheffield, United Kingdom*

Background/Introduction: Non-fracture vertebral deformities (NFDs) are common anatomical variants of the spine. They can alter the biomechanical loading of the vertebrae and may increase vertebral fracture prevalence.

Purpose: We aimed to (i) calculate the prevalence of NFDs in a patient population and (ii) ascertain whether the presence and type of NFD is associated with osteoporotic vertebral fractures (VFs).

Methods: Patients (age = 71.4 ± 11.2 years, 336 women, 134 men) with (n = 250) and without (n = 250) prevalent VFs were studied. Patients had undergone DXA and VFA (Hologic) and additional spinal imaging, if indicated, as part of their Fracture Risk Assessment (FRAS) pathway referral. An expert reader with access to VFA images only (ER) and a musculoskeletal radiologist with access to all spinal imaging (MR) used the Algorithm-Based Qualitative (ABQ) approach to identify prevalent VFs. ER also identified and characterised common NFDs using ABQ.

Agreement (Kappa (κ)) between ER and MR for VF was calculated. Relationships between NFDs and prevalent VFs were examined using Chi-squared testing (P<0.05).

Results: Per-patient agreement for VF was excellent (κ=0.940). Per-vertebra agreement ranged from κ=0.541 at T5 to κ=0.958 at L2.

Table 1
Prevalence of NFDs and relationship to prevalent VFs.

NFD Type	Prevalence in NFD group (n (%))	Prevalence in VF group (n (%))	Relationship to VFs (p value)	Relationship Type
Any NFD	212 (100)	90 (36.0)	0.004	Inverse
Osteophytes	88 (41.5)	36 (14.4)	0.06	-
Degenerative Changes	68 (32.1)	37 (14.8)	0.4	-
Schmorl's Nodes	30 (41.2)	10 (4.0)	0.06	-
Short Vertebral Height	15 (7.1)	5 (2.0)	0.2	-
Cupid's Bow	6 (2.8)	2 (0.1)	0.4	-
Scheuermann's Disease	5 (2.4)	0 (0)	0.03	Inverse

Conclusion(s): Although both VFs and NFDs occurred more frequently at the thoracolumbar junction, NFDs were inversely related to VFs. Thus, it is unlikely that NFD are an important cause of VF.

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P224 (ND)

Identification of modifier genes underlying intra-familial phenotypic variability in zebrafish OI models using whole exome sequencing (WES) and linkage analysis

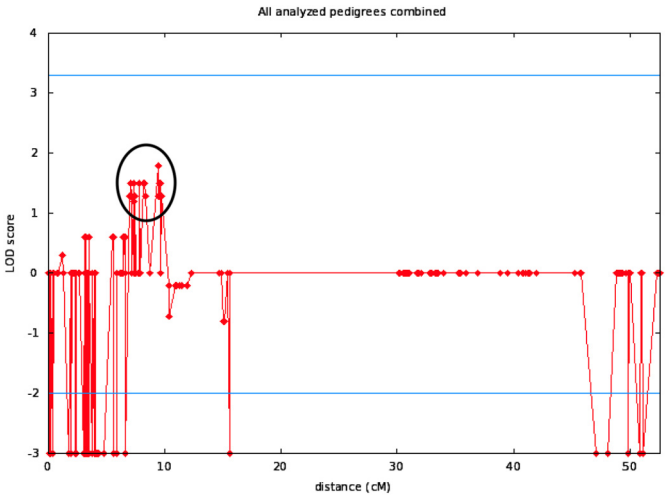
Tamara Jarayseh, Adelbert De Clercq, Mauro Milazzo, Andy Willaert, Paul Coucke
Center of Medical Genetics- Ghent University, Department of Biomolecular Medicine, Ghent, Belgium

Background/Introduction: Osteogenesis imperfecta (OI) is a rare genetic disorder with an incidence of about 1:15000 births. Skeletal deformities and bone fragility are the hallmark phenotypes. Ninety percent of the OI cases are caused by dominant mutations in the *COL1A1* or *COL1A2* genes encoding the α1- and α2-chain of type I collagen, respectively. Clinical variability in the presence of an identical causal variant is observed. This variability suggests that modifier gene(s), besides environmental factors determine phenotypic severity through a network of interactions with the causative gene.

Purpose: Zebrafish is known to be a powerful model to study skeletal disorders (Gistelinck et al, 2018). They are highly suitable to study intra-familial variability because of a high level of genomic variation and high numbers of progeny with the same causal mutation. Here, we studied an OI zebrafish model, carrying a missense substitution (Gly882Asp) in the *COL1A2* gene, and revealing an extreme phenotypic variability.

Methods: We deep-phenotyped the axial skeleton in a large number of mutants obtained from a single set of parents, by means of a combination of X-ray and Alizarin red mineral staining. Combining both methods leads to an obvious identification of mild and severely affected fish.Exome sequencing of 6 mildly and 6 severely affected *col1a2* mutants was performed on a NovaSeq 6000 illumina sequencer, followed by linkage analysis using the Superlink SNP Online tool.

Results: Whole exome sequencing followed by linkage analysis revealed a potential linked region on chromosome 14 which segregates with the phenotypic severity (figure 1). Our purpose is to further validate and narrow down this candidate region in order to identify potential modifiers.



Conclusion(s): In summary, we show that the zebrafish a powerful model for the analysis of modifier genes involved in skeletal, and most likely also in other disorders. Modifier genes represent promising targets for intervening in disease initiation and progression.

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P225 (ND)
Bone metabolism marker in an elderly patient with osteoarthritis of the knee joint

Hiroshi Yonezu
Yoshinogawa Medical Center, Orthopaedics, Yoshinogawa City, Japan

Background/Introduction: Osteoarthritis of the knee joint (knee OA) is the most common joint disease. It is suggested that bone mineral density(BMD) of proximal tibia may have a role in knee OA related pain pathogenesis. Then, a diagnosis, treatment of the osteoporosis is necessary to prevent progression of knee OA. There are some reports on BMD of patients with knee OA and osteoporosis. However, there are only a few reports on blood examinations such as those estimating the calcium (Ca), phosphorus (P), and parathyroid hormone (PTH) levels and renal function, of these patients.

Purpose: The purpose of this study was to investigate the correlation between Ca, P, PTH levels and renal function in elderly patients with knee OA and osteoporosis.

Methods: We evaluated 85 patients who had been treated for osteoporosis and knee OA (6 men, 79 women; mean age 76.1 years). Blood examinations included measurement of Ca, P, bone-specific alkaline phosphatase (BAP), tartrate-resistant acid phosphatase 5b (TRACP5b), and intact PTH levels, as well as estimated glomerular filtration rate (eGFR). BMD was measured at the level of the lumbar spine and proximal femur using dual-energy X-ray absorptiometry.

Results: TRACP5b increased with age resulting in an equilateral correlation ($r=0.228$, $p<0.05$). P increased with an increased Ca, resulting in an equalitarian correlation ($r=-0.439$, $p<0.01$). In addition, an increase in intact PTH was negatively correlated with a decrease in the Ca and P levels ($r=-0.442$, $p<0.01$ and $r=-0.506$, $p<0.01$, respectively). The average BMD was 86.1%, and 78.8% of the young adult mean (YAM) at the lumbar vertebral and proximal femoral levels, respectively.

Conclusion(s): It has been reported that a chronic increase in the PTH levels is catabolic for cortical bone. It is necessary to consider the measurement of intact PTH levels to manage osteoporosis in patients with knee OA, even if the Ca and P levels are within the normal range.

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P226 (ND)
Focal fibrocartilaginous dysplasia causing unilateral tibia vara in a child

Alexandre Bernardi[a], Maria Roberta Mezadri Bernardi[b]
[a]*Hospital Unimed, Pediatric Orthopaedics and Osteometabolism, Chapeco, Brazil*
[b]*Private practice, Nurse, Chapecó, Brazil*

Background/Introduction: To describe a rare tumor-like lesion caused by focal fibrocartilaginous of the tibia in a child.

Purpose: We described radiographic features and clinical findings in a two year old female child with a unilateral bowleg.

Methods: Case report of a rare lesion (focal fibrocartilaginous dysplasia) and its correlationship with plain radiographs and magnetic resonance images.

Results: A two year old female child with unilateral bowleg of the lower limb presents in a outpatient clinic with a severe unilateral bowleg.

Conclusion(s): Fibrocartilaginous dysplasia of the tibia is a rare tumor-like lesion with pathognomic radigraphic appearence, both in plain films or MRI. Differential diagnosis in such entity is Blount's disease, Ollier's disease, Neurofibromatosis, trauma or fibrous dysplasia.

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P227 (ND)

Knock out of tmem38b by CRISPR/Cas9 in zebrafish unveils the in vivo role of Trimeric intracellular cation (TRIC) channel B on cell homeostasis

Laura Leoni[a], Valentina Daponte[a], Francesca Tonelli[a], Roberta Gioia[a], Silvia Cotti[b], Daria Larionova[b], Eckhard Witten[b], Imke Fiedler[c], Bjorn Busse[c], Antonella Forlino[a]
[a]*University of Pavia, Department of Molecular Medicine, Pavia, Italy*
[b]*University of Ghent, Department of Biology, Ghent, Belgium*
[c]*University of Hamburg, Department of Osteology and Biomechanics, Hamburg, Germany*

Background/Introduction: *TMEM38B* encodes the endoplasmic reticulum (ER) potassium channel TRIC-B, which modulates calcium flux from the ER to the cytosol. Loss-of-function mutations in *TMEM38B* are responsible for the recessive bone disease osteogenesis imperfecta (OI) type XIV, characterized by altered collagen type I structure.

Purpose: To elucidate the link between impaired TRIC-B activity and OI, CRISPR/Cas9 was exploited to generate *tmem38b* knock out zebrafish.

Methods: *tmem38b* specific gRNA and Cas9 mRNA were injected in 1-2 cells zebrafish embryos. Mosaic fish were crossed with WT to obtain heterozygous from which homozygous were generated. Growth curve was evaluated from 5 days post fertilization (dpf) to 6 months post fertilization (mpf). Histomorphometry was performed on alizarin red stained fish. MicroCT were performed on adults to analyse bone properties. Transmission electron microscopy of skin and bone cells was carried out. The expression of the collagen specific heat shock protein Hsp47a/b was evaluated by whole mount immunohistochemistry. The experiments were approved by the Italian Ministry of Health.

Results: Two *tmem38b* zebrafish mutants were generated: one carrying a frameshift mutation resulting in a premature stop codon (*tmem38b*$^{-/-}$) and one with an in-frame deletion which eliminates Tric-b pore channel domain (*tmem38b*$^{\Delta 120\text{-}7/\Delta 120\text{-}7}$). A significant growth delay was detectable only in *tmem38b*$^{-/-}$ at 21 dpf and 1 mpf associated to reduced vertebral height and length ($p<0.05$). MicroCT analysis did not show any difference in bone volume, vertebral body thickness and vertebral body length in adult mutant fish. An increased ER cisternae size was observed in both mutants. Hsp47a/b expression was increased in mutants compared to WT (Hsp47a: WT 0%, *tmem38b*$^{-/-}$ 65%, *tmem38b*$^{\Delta 120\text{-}7/\Delta 120\text{-}7}$ 6% $p<0.05$; Hsp47b: WT 4%, *tmem38b*$^{-/-}$ 71% $p<0.05$; *tmem38b*$^{\Delta 120\text{-}7/\Delta 120\text{-}7}$ 55% $p<0.05$).

Conclusion(s): The *tmem38b* zebrafish mutants show enlargement of ER cisternae and increased expression of Hsp47 supporting a role of TRIC-B in cell homeostasis.

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Osteogenesis imperfecta and sclerostin antibody – Histological evaluation of the bone-tendon unit in the oim mouse

Antoine Chretien, Cardinal Mickaël, Catherine Behets
UCLouvain - Institute of Experimental and Clinic Research, Pole of Morphology, Brussels, Belgium

Background/Introduction: Osteogenesis imperfecta (OI), a genetic disorder of type I collagen, is characterized by bone fragility and numerous spontaneous fractures. Tendon ruptures are reported in patients but the impact of OI on bone-tendon unit (BTU) is not known.

Purpose: This preliminary study aims to describe BTU in the oim mouse, an experimental model of type III (severe) OI, and to evaluate the effect of a sclerostin neutralizing antibody (Scl-Ab). Sclerostin is a potent inhibitor of osteogenesis via inhibition of the osteoblastic Wnt pathway and we previously showed positive effects on oim bones.

Methods: Histological longitudinal sections were made through the insertions of triceps brachialis, patellar ligament and triceps suralis of oim and wild type (WT) mice treated with either Vehicle or Scl-Ab for 9 weeks [approved by ethics committee for animal care of the university]. For each BTU, five mice per group were studied. The relative bone volume (BV/TV), the length of fibrocartilage (FC) - calcified fibrocartilage (CFC) interface and the proportion of thick collagen fibers were measured with Image J software.

Results: In oim mice, epiphyseal bone showed low BV/TV ratio (0.42+/-0.1) as well as deformations. The FC/CFC interface was significantly shorter than in WT group and triceps suralis tendons contained fibrocartilage islets. Treatment with Scl-Ab enhanced BV/TV (0.63+/-0.02) and the FC/CFC interface was lenghtened by 16% ($p<0.05$) in oim mice. The proportion of thick collagen fibers in tendons and CFC of treated oim were significantly lower than those of untreated mice (-46% in tendons, $p<0.05$).

Conclusion(s): This histological study highlighted original features of oim BTU which could explain tendon ruptures in patients. Scl-Ab appeared to impact positively bone epiphysis as well as FC/CFC interface and tendon composition. Higher resolution imaging and mechanical tests would confirm these preliminary data.

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