

GENERAL ARTICLE

TrkA mediates effect of novel KIDINS220 mutation in human brain ventriculomegaly

Valerie Jacquemin^{1,†}, Mathieu Antoine¹, Sarah Duerinckx^{1,2}, Annick Massart^{1,3}, Julie Desir⁴, Camille Perazzolo¹, Marie Cassart⁵, Dominique Thomas⁵, Valérie Segers⁶, Sophie Lecomte⁶, Marc Abramowicz^{1,7,†,*} and Isabelle Pirson^{1,†}

¹IRIBHM, Université Libre de Bruxelles, 1070 Brussels, Belgium, ²Neurology Department, Hôpital Erasme, Université Libre de Bruxelles, 1070 Brussels, Belgium, ³Department of Nephrology, Hôpital Universitaire d'Anvers, 2650 Edegem, Belgium, ⁴Human Genetics Center, Institute of Pathology and Genetics, 6041 Charleroi, Belgium, ⁵Department of Gynecology and Obstetrics, Hôpitaux Iris Sud, 1050 Brussels, Belgium, ⁶Department of Anatomopathology, CHU Brugmann, 1020 Brussels, Belgium and ⁷Department of Genetic Medicine and Development, Faculty of Medicine, University of Geneva, 1211 Geneva, Switzerland

*To whom correspondence should be addressed. Tel: +44 76 226 18 22; Email: marc.abramowicz@unige.ch

Abstract

Congenital hydrocephalus is a potentially devastating, highly heterogeneous condition whose genetic subset remains incompletely known. We here report a consanguineous family where three fetuses presented with brain ventriculomegaly and limb contractures and shared a very rare homozygous variant of KIDINS220, consisting of an in-frame deletion of three amino acids adjacent to the fourth transmembrane domain. Fetal brain imaging and autopsy showed major ventriculomegaly, reduced brain mass, and with no histomorphologic abnormalities. We demonstrate that the binding of KIDINS220 to TrkA is diminished by the deletion mutation. This family is the second that associates a KIDINS220 genetic variant with human ventriculomegaly and limb contractures, validating causality of the gene and indicating TrkA as a likely mediator of the phenotype.

Introduction

Hydrocephalus is a potentially devastating condition whose long-term complications include cerebral palsy, intellectual disability, epilepsy, blindness and early death (1). Hydrocephalus is defined as a disturbance of cerebrospinal fluid (CSF) homeostasis which results in progressive ventricular dilatation due to increased CSF pressure (2). Because CSF pressure cannot readily be measured *in utero*, ventriculomegaly, i.e. the enlargement of the cerebral ventricles, is often used as a marker of fetal or congenital hydrocephalus (CH). Ventriculomegaly is the most

common central nervous system anomaly detected by prenatal ultrasound and is a sensitive indicator of CH (3). Based on its underlying origin, hydrocephalus is categorized as secondary i.e. acquired when resulting from an identified extrinsic event or primary i.e. developmental when no causal event is identified. Primary congenital hydrocephalus (PCH) is further categorized as syndromic or isolated/non-syndromic depending on the presence or absence of additional congenital anomalies. PCH has an incidence of about 0.2–0.8/1000 live births (4), and a genetic etiology can be suspected in about 40% of cases (5).

[†]These authors share senior authorship.

Received: September 11, 2020. Revised: November 9, 2020. Accepted: November 10, 2020

© The Author(s) 2020. Published by Oxford University Press. All rights reserved. For Permissions, please email: journals.permissions@oup.com

PCH is a heterogeneous pathology that encompasses a Mendelian subgroup with several inheritance modes. In humans, mutations in the L1 Cell Adhesion Molecule (*L1CAM*), and Sigma-2 Subunit (*AP1S2*) genes have been associated with X-linked PCH (6,7). Autosomal recessive inheritance has been associated with the multiple PDZ domain protein (*MPDZ*), and Coiled-Coil Domain-Containing protein 88C (*CCDC88C*) genes in several multiplex families, as well as the EMAP like 1 (*EML1*) and WD repeat domain 81 (*WDR81*) genes (8–10). More recently, enrichment with *de novo* mutations in tripartite motif 71 (*TRIM71*), SWI/SNF Related, Matrix Associated, Actin Dependent Regulator Of Chromatin Subfamily C Member 1 (*SMARCC1*), Patched-1 (*PTCH1*), Sonic Hedgehog (*SHH*) and Forkhead Box J1 (*FOXJ1*) was reported in PCH patients, strongly suggesting high penetrance and autosomal dominant inheritance (11,12). Cumulatively these genes nevertheless only explain a minority of PCH cases. In addition, one sibship of four fetuses from consanguineous parents was reported with a homozygous mutation of the Kinase D interacting substrate of 220 kDa/ankyrin-repeat-rich membrane-spanning gene (*KIDINS220/ARMS*). All fetuses presented prenatally with ventriculomegaly and limb contractures (13). Of note, heterozygous, truncating *de novo* mutations of *KIDINS220* have been associated with paraplegia, intellectual disability, nystagmus and obesity (*SINO*), a distinct syndrome albeit with ventriculomegaly as a feature (14).

KIDINS220 is a highly conserved protein within mammals, ubiquitously expressed in the central and peripheral nervous system (15), where it modulates several crucial aspects of neuronal physiology such as cell survival, differentiation into axons and dendrites and synaptic plasticity (16). It is a multifunctional scaffolding protein of 1715 amino acids composed of four transmembrane segments located in the central part of the molecule, and intracellular N- and C-termini. Its protein domains allow interactions with multiple transmembrane receptors, such as Tropomyosin-Related Kinases (*Trks*) and Nerve Growth Factor (NGF) Receptor (p75^{NTR}) (17–19), Ephrin Receptor (*Eph*) (20) and α -Amino-3-hydroxy-5-Methyl-4-isoxazolepropionic Acid Receptor (AMPA) (21), along with downstream proteins important in diverse signaling pathways (16) such as CRK like Proto-Oncogene (*CrkL*) implicated in survival signaling (19).

Trks, encoded by *NTRK1*, *NTRK2* and *NTRK3* genes in humans, constitute the neurotrophin tyrosine kinase receptor family composed of *TrkA*, *TrkB* and *TrkC*. These receptors are required for the development of the central and peripheral nervous systems. Indeed, loss of function mutations in specific *Trk* receptors have been associated with several human genetic neuropathies (22) and neurodegenerative disorders (23). Amongst these receptors, *TrkA* demonstrated the highest binding affinity for *KIDINS220* (18). *TrkA* localizes at the plasma membrane (PM) of the axon end. When activated, it triggers signaling pathways in both the axon end and neuron body (24). NGF binding to this receptor activates mitogen-activated protein kinase (MAPK) signaling responsible for neuronal differentiation and survival (25), an activity that is sustained in the presence of *KIDINS220* (18).

In this study, we identify a novel homozygous mutation of *KIDINS220* in three human fetuses from a consanguineous family of Pakistani origin who presented with ventriculomegaly and limb contractures, thereby confirming the role of *KIDINS220* in this clinical syndrome previously reported in a single sibship (13). We demonstrate that the mutation reduces the binding affinity of *KIDINS220* to *TrkA*, suggesting that PCH could result from an impaired association between *KIDINS220* and *TrkA*.

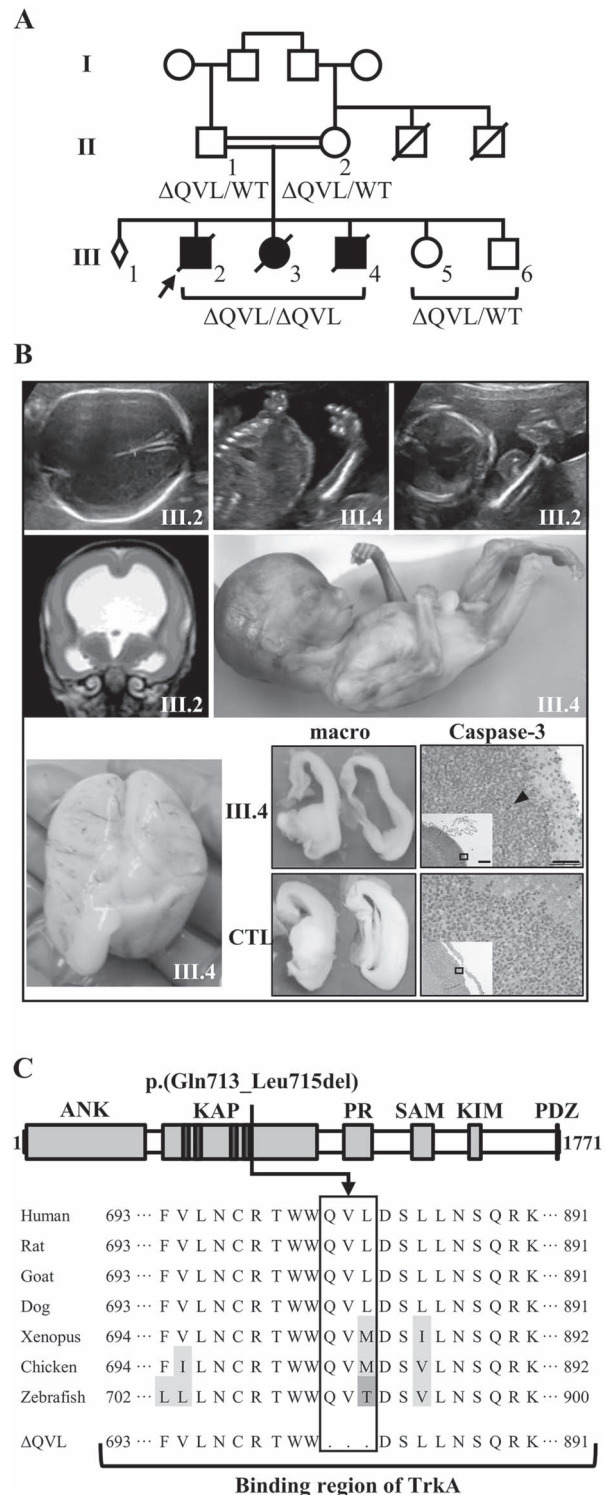


Figure 1. *KIDINS220* deletion mutation and associated phenotype. (A) Pedigree. Both parents and healthy siblings are heterozygous (Δ QVL/WT) for the *KIDINS220* in-frame deletion mutation of three amino-acids p.(Gln713_Leu715del) (c.2327_2336del). (B) Second-semester ultrasound of affected patients III.2 and III.4, showing major ventriculomegaly, clenched hands and bent wrists. Fetal autopsy showing limb contractures and reduced brain mass. Caspase-3 staining of fetal brain sections showing apoptotic cells in fetus III.4 (arrowhead, Caspase-3 immunostained cell). Full-size high-resolution images are available in supplementary data. (C) *Kidins220* protein: ANK, ankyrin

Results

A couple of first degree cousins, endured three aborted pregnancies (Fig. 1A). Severe ventriculomegaly was diagnosed as early as 14 weeks. Postmortem MRI confirmed triventricular hydrocephalus, and revealed cortical atrophy with a stratified aspect of the cortex. No gyri could be seen, suggesting lissencephaly (Fig. 1B, fetus III.2). A complete macroscopic and microscopic autopsy of fetus III.4 (Fig. 1B) revealed bilateral club feet and club hands without any other abnormalities, in particular no macrocephaly or cranial dysplasia. The neuropathological examination revealed bilateral symmetrical dilated ventricles with no other major abnormality, notably no tumoral process, hemorrhage, nor vascular malformation. There was no macroscopic or microscopic evidence of a malformation or obliteration (partial or complete) of the third ventricle, or foramina, or of the aqueduct (Fig. 1B). The choroid plexus were unremarkable. The spine showed no macroscopic malformation. In these circumstances, the etiology of the ventriculomegaly was considered as possibly developmental i.e. primary.

The whole exomes of the three affected fetuses were sequenced, and data were first analyzed for variants in 76 genes known to be implicated in isolated or syndromic human hydrocephalus (Supplementary Material, Table S1). Variants were filtered for a minor allelic frequency (MAF) depending on the inheritance mode, respectively MAF < 2% for autosomal recessive inheritance or MAF < 0.5% for autosomal dominant and X-linked inheritance. No variant was found that cosegregated with the phenotype. As the three fetuses also presented limb contractures, a list of 267 genes associated with arthrogryposis was analyzed as above (Supplementary Material, Table S2), and again no variant was found that cosegregated with the phenotype.

We then filtered the exome data for rare variants found homozygous in the three affected fetuses and heterozygous in the parents, which revealed two genes. One variant, c.61C > T; p.(Arg21Trp) was found in the synaptotagmin-like 2 gene, SYTL2 (NM_206927.2). The other variant, c.2137_2145del; p.(Gln713_Leu715del) was found in KIDINS220 (NM_020738.3). SYTL2 encodes a protein of the immunological synapse (26), which is regarded as the T cell homolog of a primary cilium (27). Although hydrocephalus is a feature of some ciliopathies, the low protein expression of SYTL2 in the brain, made it an unlikely candidate (<https://www.proteinatlas.org/>). Conversely, KIDINS220 appeared as a strong candidate, given the previously reported family (11) and a knockout mouse model in which embryos displayed progressive dilatation of cerebral ventricles (28).

KIDINS220 sequence is highly conserved (more than 90% protein sequence homology) within mammals. Very strong conservation is evident in the stretch of amino-acids downstream of the fourth and last transmembrane domain (17), where the variant is located (Fig. 1C). The fourth transmembrane domain and adjacent amino acids have been shown to interact with TrkA (17–19). The KIDINS220 variant in the present family, consisted of an in-frame deletion in exon 17, located on chr2:g.8925956_8925964del. In the protein, this mutation removes the Glutamine at position 713, the Valine at position 714 and the Leucine at position 715; p.(Gln713_Leu715del)

(Fig. 1C). The variant is encompassed in a 1.5 Mb homozygous stretch (chr2:8439905–9983716), consistent with homozygosity by descent in the three fetuses. The variant was absent from the GnomAD database (29). The gene is classified as intolerant to variation in RVIS (score = –2.2) (30) meaning that only 1.3% of the coding genome is more intolerant to variation. As the variant removed the three amino acids adjacent to the fourth transmembrane domain, previously shown to interact with TrkA, we hypothesized that this deletion would impact the interaction between KIDINS220 and TrkA.

To address this question, we performed co-immunoprecipitation experiments in HEK293 cells. We coexpressed TrkA with a FLAG-tagged KIDINS220 wild-type (WT) protein or a FLAG-tagged KIDINS220 Δ QVL harboring the same mutation as observed in the proband. Whole cell extracts (WCE) were first analyzed for protein expression (Fig. 2A, WCE lane). Consistent with its N-glycosylation maturation process, we observed three forms of TrkA i.e. the 75 kDa immature form 3, the 100 kDa intermediate form 2 and the 140 kDa mature form 1 (31). Interaction between Kidins220 and mature TrkA is known to result in effective transmembrane signaling (18,31). Therefore, using a PM purification protocol (32), we achieved more than 2-fold enrichment of the mature form of TrkA (Fig. 2A). FLAG-TRAP immunoprecipitations were performed on PM fractions and we looked for the co-immunoprecipitated mature TrkA (Fig. 2B). We observed a decrease of 38% in the amount of mature TrkA bound to the mutated KIDINS220 (Δ QVL) as compared to the WT protein (Fig. 2C). In the absence of enrichment, the same trend was observed but failed to reach significance (Supplementary Material, Fig. S1).

Knock-out Kidins220 $^{-/-}$ mice have been reported with slightly increased neuronal cell death throughout the brain, mainly in the germinative zone (28) without any increase in the proliferation rates. We, thus, complemented the autopsy of fetus III.4 with Caspase-3 (Fig. 1B) and Ki67 (Supplementary Material, Fig. S2) immunohistological stainings on 4 mm brain slices. Hematoxylin-eosine saffron (HES) staining was used to assess the integrity of cerebral structures. An unrelated 16 week fetus with a normal brain was used as control. Unfortunately, Caspase-3 and Ki67 immunostaining failed to show a significant difference between patient and control, possibly linked to tissue autolysis after fetal demise. HES staining showed the integrity of the cortical plate in the affected fetus with no histological signs of a destructive or degenerative process (Supplementary Material, Fig. S2).

Discussion

We report on a consanguineous family, in which three fetuses presented ventriculomegaly and limb contractures, fitting a previously described phenotype (13). As this phenotype had only been reported in a single-family with a KIDINS220 variant, our study validates the causality of KIDINS220 beyond a reasonable doubt. Whole exome sequencing (WES) performed on the three fetuses and both parents, allowed for the identification of a co-segregating homozygous in-frame deletion in exon 17 of KIDINS220. Linkage analysis of this extremely rare variant, unreported in GnomAD, with the phenotype in the three

repeat 4-396aa; KAP, KAP NTPase domain 440-953aa; TM, transmembrane domains 500-520aa, 525-545aa, 660-680aa, 686-706aa; PR, proline-rich stretch 1057-1151aa; SAM, Sterile alpha motif domain 1211-1278aa; KIM kinase light chain (KLC)-interacting motif 1406-1445aa; PDZB PDZ binding c-terminus, 1768-1771aa. The known TrkA binding region is shown. Residues highlighted in light gray indicate the conservation of amino acids with strongly similar chemical properties. Residue highlighted in dark grey indicates amino acids with different chemical properties. The deletion mutation is boxed.

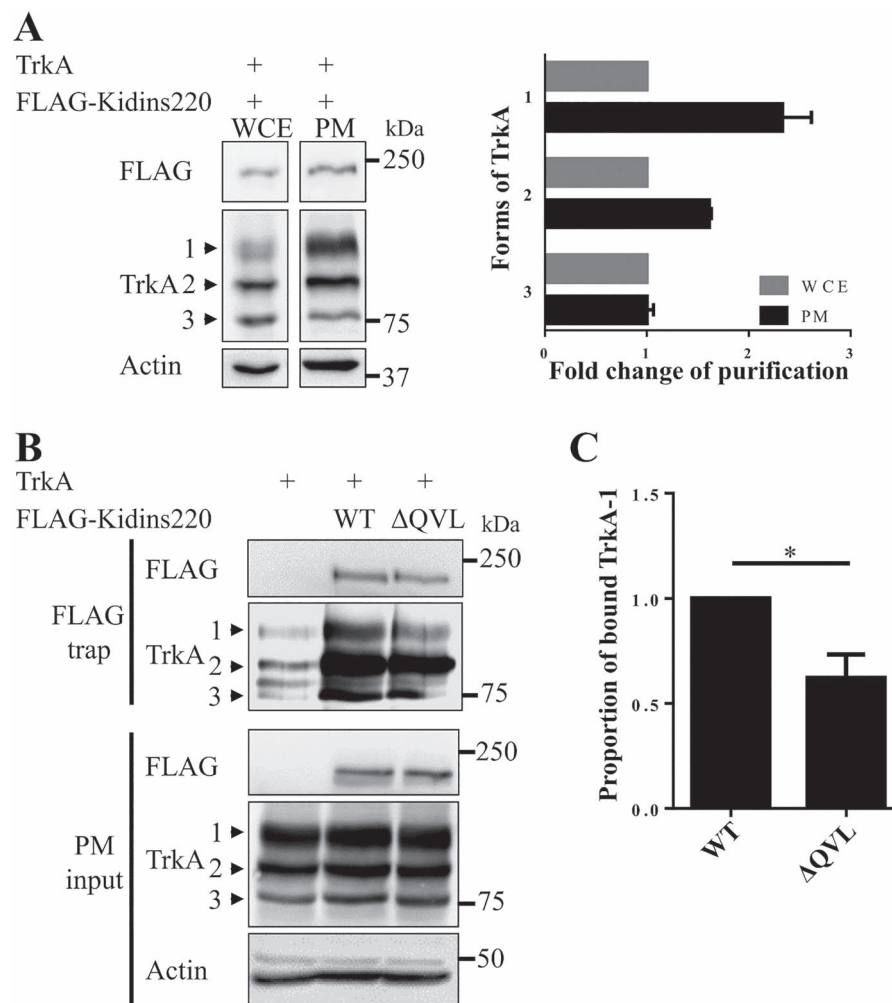


Figure 2. The deletion mutation impairs the binding of KIDINS220 to TrkA. (A, B) HEK293 cells were transfected with 2 μ g of TrkA and 2 μ g of either FLAG-tagged WT KIDINS220 or mutated KIDINS220 (Δ QVL). (A) (left) Cell lysate of transfected condition TrkA and WT KIDINS220 before (WCE) and after (PM) plasma membrane protein purification. Transfected and endogenous protein expression were immunoblotted with the indicated antibodies. (right) Quantification of the different forms of TrkA before (WCE) and after (PM) plasma membrane protein purification measured on three independent experiments (B) PM fractions immunoprecipitated by FLAG-trap and blotted with indicated antibodies. Equal protein loading and expression were verified by western Blot using indicated antibodies (PM fraction input) (C) Quantification of bound mature TrkA to KIDINS220 Δ QVL compared to KIDINS220 WT ($n=6$). Raw data were analyzed using a paired t-test ($P=0.0291$). The proportion of bound TrkA-1 was analyzed using a one-sample t-test ($P=0.0183$) and represented with mean $\pm$ SEM. The ratio of TrkA-1/FLAG-KIDINS220 (Δ QVL/WT) was measured with ImageJ software. * $P \leq 0.05$.

consanguineous fetuses from first-cousin parents, produces a LOD score of 2.4, which *per se* is strongly indicative of linkage to the locus (33). No significant variant was found in other known ventriculomegaly or hydrocephalus gene, nor in other genes of the exome. In particular, the only other variant found homozygous and concordant in the three fetuses was in a gene not involved in the phenotype and not expressed in the brain or muscle.

KIDINS220/ARMS, encoded by KIDINS220, is a protein with multiple protein interaction domains. The N-terminal region mediates rearrangements of the actin cytoskeleton triggered by NGF signaling (34). The C-terminal tail contains several residues subjected to posttranslational modifications and regions modulating the binding to molecular interactors amongst which adaptor proteins of the MAPKinase pathway (35), a KIM motif involved in the localization of the protein at the neurite (36) and finally, a PDZ motif which is responsible for KIDINS220 localization at the neuromuscular junction (NMJ) (20).

The in-frame deletion variant identified in the present family affected a region of KIDINS220 known to interact with TrkA (18). Through NGF activation, the TrkA receptor induces the recruitment of different signaling and adaptor proteins to trigger the PI3Kinase-AKT, PLC γ 1 and Ras-MAPKinase pathways. Indeed, after its phosphorylation by TrkA, KIDINS220 mediates TrkA signaling toward sustained MAPkinase activity, which is closely associated with neuronal survival, neurite outgrowth and differentiation (18,35). Consistent with these data, the knock-down of KIDINS220 was shown to decrease ERK activation and neuronal viability and to enhance excitotoxic cell death (37,38). On the other hand, cells transfected with a TrkA specific siRNA showed a significant down-regulation in the expression of proteins such as pERK1/2, while there was an upregulation of activated caspase-3 and pJNK, which mediate cell death (39,40). Moreover, Kidins220 knockout mice embryos showed, among other features, impairment in the neuronal responses toward neurotrophic stimuli and a high degree of neuronal cell death in

the CNS leading to enlargement of the ventricles (41). The lesser binding affinity to TrkA conveyed by the in-frame deletion mutation of KIDINS220 could hence result in impaired survival signals. Interestingly, a defect in stem cell neurogenesis in the ventricular zone, rather than the active accumulation of CSF, seems to be involved in a subgroup of PCH (11). This is in line with the autopsy findings showing a reduction in cerebral parenchyma volume in addition to ventriculomegaly, and questionably, a trend toward increased Caspase-3 on immunohistology of the fetal brain autopsy in patient III.4, shown by the presence of some apoptotic cells in the ventricular zone (Fig. 1B).

Given the multiscaffolding nature of KIDINS220, identifying the mechanisms implicated in the observed phenotype remains challenging. The mutation was located in a region of KIDINS220 known to interact with TrkA, and we provide robust data for the implication of TrkA in mediating its effect in CH. The possible role of other signaling pathways in the observed phenotype could, however, not be ruled out. Moreover, it is theoretically possible that SYTL2 would participate in a digenic manner alongside KIDINS220 in the observed phenotype (42–44).

In addition to ventriculomegaly, all fetuses presented limb contractures. It is known that KIDINS220 and TrkB, another Trk receptor, are diffusely distributed on the sarcolemma of skeletal muscles and progressively concentrate at the NMJ during development (20). TrkB was shown to play an important role to sustain NMJ function (45) and, like TrkA, displayed the ability to phosphorylate KIDINS220 which plays a role in different signaling pathways (17). Moreover, conditional knockout mice for ERK 1/2 in muscular fibers demonstrated the impact of the MAP kinase pathway in the development and integrity of the NMJ (46). We can, therefore, speculate that a defective interaction of KIDINS220 with TrkB, as we observed with TrkA, could hamper TrkB signaling at the NMJs through the MAPKinase pathway. We can however not exclude that the limb contractures may result from brain malfunction associated with ventriculomegaly (47).

The homozygous in-frame deletion within the TrkA binding domain of KIDINS220 produced the same phenotype as observed with a total loss of the protein (13). This supports the importance of KIDINS220 tight regulation for proper neuronal development (48) and underscores the importance of TrkA signaling mediated by KIDINS220. It also indicates that the present deletion mutation is a loss of function mutation, which is consistent with an autosomal recessive mode of inheritance and asymptomatic heterozygous parents.

Ventriculomegaly is a frequent finding on routine prenatal ultrasounds (3). In a time where molecular testing is offered postnatally or even prenatally for a growing number of conditions, we recommend that KIDINS220 should be added to diagnostic gene panels analyzed for mutations in patients with PCH/ventriculomegaly and/or limb contractures.

In summary, our observations validate KIDINS220 biallelic loss of function mutations as a cause of the human autosomal recessive phenotype of fetal ventriculomegaly and limb contractures and show that decreased TrkA binding is involved in mediating the phenotype.

Materials and Methods

Patients

A couple of Pakistani origin, who are first degree cousins, presented a history of three fetuses affected with ventriculomegaly and limb contractures. Both parents had unremarkable medical histories, normal head sizes and normal intellects. Pregnancies

of the three affected fetuses followed a normal early course, with no report of alcohol, or drug abuse, nor infection. The couple also presented, previously to the three affected fetuses, a first non-progressing pregnancy (III.1). In the second fetus (III.2), ultrasound anomalies reported at 22 weeks of pregnancy included major triventricular hydrocephalus with septal disruption and clubfeet. The couple chose to terminate the pregnancy. Fluorescence in situ hybridization (FISH) of chromosomes X (centromere Xp11.1-q11.1 (DXZ1)/chromosome Y, 21 (locus 21q22.13), 13 (locus 13q14.2) and 18 (centromere 18p11.1-q11.1 (D18Z1) was normal. CGH array analysis in the first fetus was normal and the family was referred to the human genetics research unit for further testing. In fetus III.3, echographic anomalies were observed at 14 weeks of pregnancy with major hydrocephalus and clubfeet. The septum was in place. The couple opted for elective termination of the pregnancy. In fetus III.4, ultrasound anomalies were reported at 15 weeks of pregnancy with major ventriculomegaly, clubfeet and clubhands. After informed consent, DNA was extracted from cultured amniotic fluid cells (III.2) or from umbilical cord cells (III.3 and III.4) and WES was performed. The couple later bore two healthy children (III.5 and III.6). The pregnancy of III.5 was unremarkable, with a normal fetal MRI performed at 31 weeks after ultrasound scanning had revealed a small ventricular asymmetry. Fetal imaging revealed an isolated ventricular asymmetry within normal limits. III.6's pregnancy, in which prenatal DNA analysis was performed, revealed heterozygosity of the fetus for the KIDINS220 mutation.

Fetal autopsy and immunostaining of fetal brain

A complete medical autopsy examination of fetus III.4 was performed. The neuropathological examination included histological stainings: HES (hematoxylin-eosin Safran following Mayer protocol) and Cresyl-Violet as well as immunohistological stainings: Ki67 30.0790–42867 (Roche, Bale, Switzerland) performed within the pathology department using the Roche Benchmark II and an anti-Caspase-3 EPR18297 (Abcam, Cambridge, UK) staining performed at the Institut of Pathology and Genetics in Gosselies, Belgium using Dako Omnis (Agilent Technologies, California, USA). Images were obtained using the Nikon eclipse Gi coupled with Sony camera DFK72AUC02, and analyzed with NIS Element-D program.

Whole exome sequencing

For WES, genomic DNA from the patients were sheared, exonic sequences were captured using the SeqCap EZ Human Exome v3.0 (64 Mb) kit (Roche®) and read on 100 base pairs on each side ('paired-end') by the HiSeq 1500 Illumina sequencer at the BRIGHTcore Brussels Interuniversity Genomics High Throughput core (49), with a minimal average depth coverage of 75X. Raw sequences were aligned to the reference genome GRCh37 using BWA algorithm version 0.7.15 (50), duplicated reads were then marked using Picard version 1.97 (51), alignment quality was improved using the GATK (52) realigner and base recalibrator version 2.7 and finally, variants were called using GATK Haplotype Caller version 2.7. The resulting variant set was annotated and filtered using the Highlander software (53). Variants were filtered for quality criteria (pass GATK standard filter, read depth >5, variant confidence by depth ≥10), allelic frequency <0.5% (based on the maximum minor allele frequency found in ExAC (54), 1000G (55), ESP6500 (56), gnomAD (57), ARIC5606 (58) and our in-house database), non-synonymous or

splice junction effect in protein coding genes (using biotype from Ensembl (59) and snpeff_effect from SnpEff (60)), and genotype (homozygous or heterozygous variants in a subset of syndromic or non-syndromic hydrocephalus genes extracted from the literature (Supplementary Material, Table S1), arthrogryposis gene list (Supplementary Material, Table S2) and shared homozygous or biallelic variants from all other genes of the exome). Variants were then sorted by decreasing Consensus Prediction Score, corresponding to a combination of predictors defined by Highlander software. One point was given for each of the six prediction softwares (Mutation Taster, Sift, Polyphen2, LTR, Mutation Assessor, FATHMM) when the variant is predicted to be pathogenic. Additional points can be given based on the type of mutation (snpeff_effect). The coverage of the gene panels in the exome was very satisfying.

In the isolated or syndromic human hydrocephalus gene panel, 63 genes had a coverage above 20x higher than 80% of the gene exons. Six genes (CRB2, FGFR3, FKR, KIF7, MAF, PIK3R2, SKI) had a coverage above 20x higher than 70%, and two genes (TBX1, ZIC2) higher than 60%.

In the arthrogryposis gene panel, 233 genes had a coverage above 20x higher than 80% of the gene exons. Twenty-two genes (ADATSL2, ALG2, CD6, EBP, EMD, FAM20C, FGD1, FGFR3, FKR, GCK, HOXA13, KIF7, NEFH, NOG, PITX1, PLEKHG5, SHOX, SKI, SOX9, TREX1, WNT5A, ZBTB42) had a coverage above 20x higher than 70%, and two genes (MNX1, TWIST2) higher than 60%. One gene, CHST3, had a coverage above 20x only higher than 50% but a coverage above 10x higher than 70% of the gene exons. Three genes (ARX, COL11A2, NEU1) had a coverage above 20x lower than 40%, but still had a coverage above 1x higher than 85%.

Cell cultures and transfections

p.CMV5-TrkA was a kind gift from Juan Carlos Arévalo (New York University School of Medicine). p.FLAG-ARMS was purchased from Addgene (#24086) hereafter referred to as FLAG-Kidins220 (WT). The plasmid containing the mutation was generated by Eurofins Genomics (Ebersberg, Germany) and hereafter referred to as FLAG-Kidins220 Δ QVL.

HEK293 cells were cultured in Dulbecco's modified Eagle's medium (Life Technologies Limited) supplemented with 10% fetal bovine serum (Life Technologies Limited), 50 units/ml penicillin (Life Technologies Limited), 50 μ g/ml streptomycin (Life Technologies Limited), 2.5 μ g/ml fungizone (Life Technologies Limited) and 1 mM Na pyruvate (Life Technologies Limited). For transfection, HEK293 cells were seeded in 10 cm plates (2.2×10^6 cells) and transfected with 2 μ g of p.CMV5-TrkA, together with either 2 μ g of FLAG-Kidins220, or FLAG-Kidins220 Δ QVL using XtremeGENE9 reagent (Roche Diagnostics, Basel, Switzerland) as recommended by the manufacturer.

Western blotting, co-immunoprecipitation and antibodies

The following antibodies were used for western blot: mouse TrkA monoclonal sc7268 (Santa Cruz, Dallas, TX) and rabbit FLAG monoclonal PA1-984B (Thermo Fischer Scientific, Waltham, MA). HRP-linked anti-mouse #7076 and HRP-linked anti-rabbit #7074 (Cell Signaling Technology, Leiden, The Netherlands) were used as secondary antibodies for ECL detection with western lightning Plus-ECL solutions (PerkinElmer, Waltham, MA). The membranes were detected using Fusion Solo S imaging system (Vilber Lourmat, Marne-La-Vallée, France).

For western blot analysis, the culture medium was removed, and the cells were washed in cold phosphate-buffered saline and placed in either lysis buffer (25 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA pH 7.5, 0.5% NP40, 0.2 mM orthovanadate, 50 mM NaF, and cocktail inhibitors from ROCHE) or purification buffer (25 mM Tris-HCl pH 8, 0.5 mM orthovanadate, 5 mM NaF, 0.25 mM DTT and cocktail inhibitors from ROCHE). The purification steps for the preparation of PM fraction were followed, as previously described (32). Proteins were denaturated by boiling for 3 min in Laemmli Buffer 1X (65 mM Tris-HCl pH 6.8, 2% SDS, 5% glycerol, 0.01% bromophenol blue, 0.1% DTT). Proteins were separated using SDS-PAGE, followed by western blotting and revealed by ECL detection with the western lightning Plus system (PerkinElmer, Waltham, MA).

For co-immunoprecipitation experiments, cells were lysed as described above, equal amounts of protein were engaged with ANTI-FLAG M2 Affinity Gel (Sigma-Aldrich, Saint-Louis, MO) for 3 h at 4°C. The complexes were washed three times in buffer solution (25 mM Tris-HCl pH 8, 150 mM NaCl, NP40 0.5%), denaturated and boiled for 5 min in Laemmli Buffer 2X. Proteins were separated using SDS-PAGE, followed by western blotting and revealed by ECL detection with the western lightning Plus system (PerkinElmer, Waltham, MA).

Quantification and statistic analysis

Quantifications of western blot signals were done using ImageJ software. Background signal was subtracted from measured signals of TrkA, KIDINS220 and KIDINS220 Δ QVL. The Intensity of the bound TrkA signal was normalized by the signal intensity of either the immunoprecipitated KIDINS220 or KIDINS220 Δ QVL. Both ratios were then compared. GraphPad Prism software was used for statistical analysis using the corresponding t-test (no significance (ns) $P > 0.05$; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$).

Supplementary data

Supplementary Material is available at HMG Online.

Ethical statement

All procedures complied with the ethical guidelines of Hôpital Erasme—Université Libre de Bruxelles, whose Ethics Committee approved our study protocol under reference P2019/598 (Ethics Committee Erasme Hospital).

Acknowledgements

We thank Dr Anais Drielsma for her help in the collection of DNA samples, and Juan Carlos Arevalo for providing us with the TrkA plasmid. We thank the Anatomopathology Department of the Institut of Pathology and Genetics (IPG) for their help with caspase-3 staining. Finally, we are grateful to the parents who agreed to participate in this study.

Conflict of Interest statement. None declared.

Funding

This work was supported by a Jean Van Damme Orphan Disease grant of the Fonds Erasme and an Action de Recherche Concertée (ARC) of the Belgian Fédération Wallonie-Bruxelles, as well as the Belgian's Kid Fund of Hôpital Universitaire des Enfants Reine Fabiola.

References

- Persson, E.-K., Anderson, S., Wiklund, L.-M. and Uvebrant, P. (2007) Hydrocephalus in children born in 1999-2002: epidemiology, outcome and ophthalmological findings. *Childs Nerv. Syst.*, **23**, 1111-1118.
- Mori, K., Shimada, J., Kurisaka, M., Sato, K. and Watanabe, K. (1995) Classification of hydrocephalus and outcome of treatment. *Brain and Development*, **17**, 338-348.
- Nagaraj, U.D. and Kline-Fath, B.M. (2020) Imaging diagnosis of ventriculomegaly: fetal, neonatal, and pediatric. *Childs Nerv. Syst.*, **36**, 1669-1679.
- Chi, J.H., Fullerton, H.J. and Gupta, N. (2005) Time trends and demographics of deaths from congenital hydrocephalus in children in the United States: National Center for Health Statistics data, 1979 to 1998. *J. Neurosurg.*, **103**, 113-118.
- Verhagen, J.M., Schrander-Stumpel, C.T., Krapels, I.P., de Die-Smulders, C.E.M., van Lint, F.H.M., Willekes, C., Weber, J.W., Gavilanes, A.W.D., Macville, M.V.E. et al. (2011) Congenital hydrocephalus in clinical practice: a genetic diagnostic approach. *Eur. J. Med. Genet.*, **54**, e542-e547.
- Rosenthal, A., Jouet, M. and Kenwrick, S. (1992) Aberrant splicing of neural cell adhesion molecule L1 mRNA in a family with X-linked hydrocephalus. *Nat. Genet.*, **2**, 107-112.
- Tarpey, P.S., Stevens, C., Teague, J., Edkins, S., O'Meara, S., Avis, T., Barthorpe, S., Buck, G., Butler, A., Cole, J. et al. (2006) Mutations in the gene encoding the sigma 2 subunit of the adaptor protein 1 complex, AP1S2, cause X-linked mental retardation. *Am. J. Hum. Genet.*, **79**, 1119-1124.
- Al-Dosari, M.S., Al-Owain, M., Tulbah, M., Kurdi, W., Adly, N., Al-Hemidan, A., Masoodi, T.A., Albash, B. and Alkuraya, F.S. (2013) Mutation in MPDZ causes severe congenital hydrocephalus. *J. Med. Genet.*, **50**, 54-58.
- Drielsma, A., Jalas, C., Simonis, N., Désir, J., Simanovsky, N., Pirson, I., Elpeleg, O., Abramowicz, M. and Edvardson, S. (2012) Two novel CCDC88C mutations confirm the role of DAPLE in autosomal recessive congenital hydrocephalus. *J. Med. Genet.*, **49**, 708-712.
- Shaheen, R., Sebai, M.A., Patel, N., Ewida, N., Kurdi, W., Altweijri, I., Sogaty, S., Almadawi, E., Seidahmed, M.Z., Alnemri, A. et al. (2017) The genetic landscape of familial congenital hydrocephalus: congenital hydrocephalus. *Ann. Neurol.*, **81**, 890-897.
- Furey, C.G., Choi, J., Jin, S.C., Zeng, X., Timberlake, A.T., Nelson-Williams, C., Mansuri, M.S., Lu, Q., Duran, D., Panchagnula, S. et al. (2018) De novo mutation in genes regulating neural stem cell fate in human congenital hydrocephalus. *Neuron*, **99**, 302-314.
- Wallmeier, J., Frank, D., Shoemark, A., Nöthe-Menchen, T., Cindric, S., Olbrich, H., Loges, N.T., Aprea, I., Dougherty, G.W., Pennekamp, P. et al. (2019) De novo mutations in FOXJ1 result in a motile Ciliopathy with hydrocephalus and randomization of left/right body asymmetry. *Am. J. Hum. Genet.*, **105**, 1030-1039.
- Mero, I.-L., Mørk, H.H., Sheng, Y., Blomhoff, A., Opheim, G.L., Erichsen, A., Vigeland, M.D. and Selmer, K.K. (2017) Homozygous KIDINS220 loss-of-function variants in fetuses with cerebral ventriculomegaly and limb contractures. *Hum. Mol. Genet.*, **26**, 3792-3796.
- Josifova, D.J., Monroe, G.R., Tessadori, F., de Graaff, E., van der Zwaag, B., Mehta, S.G., Harakalova, M., Duran, K.J., Savelberg, S.M.C., Nijman, I.J. et al. (2016) Heterozygous KIDINS220/ARMS nonsense variants cause spastic paraplegia, intellectual disability, nystagmus, and obesity. *Hum. Mol. Genet.*, **25**, 2158-2167.
- Scholz-Starke, J. and Cesca, F. (2016) Stepping out of the shade: control of neuronal activity by the scaffold protein Kidins220/ARMS. *Front. Cell. Neurosci.*, **10**.
- Neubrand, V.E., Cesca, F., Benfenati, F. and Schiavo, G. (2012) Kidins220/ARMS as a functional mediator of multiple receptor signalling pathways. *J. Cell Sci.*, **125**, 1845-1854.
- Kong, H., Boulter, J., Weber, J.L., Lai, C. and Chao, M.V. (2001) An evolutionarily conserved Transmembrane protein that is a novel downstream target of Neurotrophin and Ephrin receptors. *J. Neurosci.*, **21**, 176-185.
- Arévalo, J.C., Yano, H., Teng, K.K. and Chao, M.V. (2004) A unique pathway for sustained neurotrophin signalling through an ankyrin-rich membrane-spanning protein. *EMBO J.*, **23**, 2358-2368.
- Chang, M.-S., Arevalo, J.C. and Chao, M.V. (2004) Ternary complex with Trk, p75, and an ankyrin-rich membrane spanning protein. *J. Neurosci. Res.*, **78**, 186-192.
- Luo, S., Chen, Y., Lai, K.-O., Arévalo, J.C., Froehner, S.C., Adams, M.E., Chao, M.V. and Ip, N.Y. (2005) α -Syntrophin regulates ARMS localization at the neuromuscular junction and enhances EphA4 signaling in an ARMS-dependent manner. *J. Cell Biol.*, **169**, 813-824.
- Arévalo, J.C., Wu, S.H., Takahashi, T., Zhang, H., Yu, T., Yano, H., Milner, T.A., Tessarollo, L., Ninan, I., Arancio, O. and Chao, M.V. (2010) The ARMS/Kidins220 scaffold protein modulates synaptic transmission. *Mol. Cell. Neurosci.*, **45**, 92-100.
- Reichardt, L.F. (2006) Neurotrophin-regulated signalling pathways. *Philos. Trans. R. Soc. B Biol. Sci.*, **361**, 1545-1564.
- Gupta, V.K., You, Y., Gupta, V.B., Klistorner, A. and Graham, S.L. (2013) TrkB receptor signalling: implications in neurodegenerative, psychiatric and proliferative disorders. *Int. J. Mol. Sci.*, **14**, 10122-10142.
- Haddad, Y., Adam, V. and Heger, Z. (2017) Trk receptors and Neurotrophin cross-interactions: new perspectives toward manipulating therapeutic side-effects. *Front. Mol. Neurosci.*, **10**.
- Novak, K. (2004) Getting on TRK. *Nat. Rev. Cancer*, **4**, 920.
- Holt, O., Kanno, E., Bossi, G., Booth, S., Daniele, T., Santoro, A., Arico, M., Saegusa, C., Fukuda, M. and Griffiths, G.M. (2008) Slp1 and Slp2-a localize to the plasma membrane of CTL and contribute to secretion from the immunological synapse. *Traffic*, **9**, 446-457.
- Cassio, C. and Baldari, C.T. (2019) A Ciliary view of the immunological synapse. *Cell*, **8**, 789.
- Cesca, F., Yabe, A., Spencer-Dene, B., Arrigoni, A., Al-Qatari, M., Henderson, D., Phillips, H., Koltzenburg, M., Benfenati, F. and Schiavo, G. (2011) Kidins220/ARMS is an essential modulator of cardiovascular and nervous system development. *Cell Death Dis.*, **2**, e226.
- Karczewski, K.J., Francioli, L.C., Tiao, G., Cummings, B.B., Alföldi, J., Wang, Q., Collins, R.L., Laricchia, K.M., Ganna, A., Birnbaum, D.P. et al. (2020) The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature*, **581**, 434-443.
- Genic Intolerance. Genic intolerance <http://genic-intolerance.org/about.jsp> (accessed May 31, 2019).
- Jullien, J., Guili, V., Reichardt, L.F. and Rudkin, B.B. (2002) Molecular kinetics of nerve growth factor receptor trafficking and activation. *J. Biol. Chem.*, **277**, 38700-38708.
- Yamamoto, N., Yamashita, Y., Yoshioka, Y., Nishiumi, S. and Ashida, H. (2016) Rapid preparation of a plasma membrane fraction: western blot detection of Translocated glucose

- transporter 4 from plasma membrane of muscle and adipose cells and tissues. *Curr. Protoc. Protein Sci.*, **85**, 29.18.1–29.18.12.
33. Terwilliger, J.D. and Ott, J. (1994) Handbook of Human Genetic Linkage. In *Handbook of Human Genetic Linkage*. Johns Hopkins University Press, Baltimore (Md.).
 34. Neubrand, V.E., Thomas, C., Schmidt, S., Debant, A. and Schiavo, G. (2010) Kidins220/ARMS regulates Rac1-dependent neurite outgrowth by direct interaction with the RhoGEF trio. *J. Cell Sci.*, **123**, 2111–2123.
 35. Arévalo, J.C., Waite, J., Rajagopal, R., Beyna, M., Chen, Z.-Y., Lee, F.S. and Chao, M.V. (2006) Cell survival through Trk neurotrophin receptors is differentially regulated by ubiquitination. *Neuron*, **50**, 549–559.
 36. Bracale, A., Cesca, F., Neubrand, V.E., Newsome, T.P., Way, M. and Schiavo, G. (2007) Kidins220/ARMS is transported by a Kinesin-1-based mechanism likely to be involved in neuronal differentiation. *Mol. Biol. Cell*, **18**, 142–152.
 37. Kumar, V., Gupta, A.K., Shukla, R.K., Tripathi, V.K., Jahan, S., Pandey, A., Srivastava, A., Agrawal, M., Yadav, S., Khanna, V. and Pant, A.B. (2015) Molecular mechanism of switching of TrkA/p75 NTR Signaling in Monocrotophos induced neurotoxicity. *Sci. Rep.*, **5**, 14038.
 38. López-Menéndez, C., Gascón, S., Sobrado, M., Vidaurre, O., Higuero, A., Rodríguez-Peña, A., Iglesias, T. and Díaz-Guerra, M. (2009) Kidins220/ARMS downregulation by excitotoxic activation of NMDARs reveals its involvement in neuronal survival and death pathways. *J. Cell Sci.*, **122**, 3554–3565.
 39. Friedman, W.J. (2000) Neurotrophins induce death of hippocampal neurons via the p75 receptor. *J. Neurosci.*, **20**, 6340–6346.
 40. Yoon, S.O., Casaccia-Bonnel, P., Carter, B. and Chao, M.V. (1998) Competitive Signaling between TrkA and p75 nerve growth factor receptors determines cell survival. *J. Neurosci.*, **18**, 3273–3281.
 41. Cesca, F., Yabe, A., Spencer-Dene, B., Scholz-Starke, J., Medrihan, L., Maden, C.H., Gerhardt, H., Orriss, I.R., Baldelli, P., Al-Qatari, M. et al. (2012) Kidins220/ARMS mediates the integration of the neurotrophin and VEGF pathways in the vascular and nervous systems. *Cell Death Differ.*, **19**, 194–208.
 42. Duerinckx, S., Jacquemin, V., Drunat, S., Vial, Y., Passemard, S., Perazzolo, C., Massart, A., Soblet, J., Racapé, J., Desmyter, L. et al. (2020) Digenic inheritance of human primary microcephaly delineates centrosomal and non-centrosomal pathways. *Hum. Mutat.*, **41**, 512–524.
 43. Ishida, M., Cullup, T., Boustred, C., James, C., Docker, J., English, C., Lench, N., Copp, A.J., Moore, G.E., Greene, N.D.E. and Stanier, P. (2018) A targeted sequencing panel identifies rare damaging variants in multiple genes in the cranial neural tube defect, anencephaly. *Clin. Genet.*, **93**, 870–879.
 44. Kim, A., Savary, C., Dubourg, C., Carré, W., Mouden, C., Hamdi-Rozé, H., Guyodo, H., Le Douce, J., Pasquier, L., Flori, E. et al. (2019) Integrated clinical and omics approach to rare diseases: novel genes and oligogenic inheritance in holoprosencephaly. *Brain*, **142**, 35–49.
 45. Lanuza, M.A., Just-Borràs, L., Hurtado, E., Cilleros-Mañé, V., Tomàs, M., Garcia, N. and Tomàs, J. (2019) The impact of kinases in amyotrophic lateral sclerosis at the neuromuscular synapse: insights into BDNF/TrkB and PKC Signaling. *Cell*, **8**, 1578.
 46. Seaberg, B., Henslee, G., Wang, S., Paez-Colasante, X., Landreth, G.E. and Rimer, M. (2015) Muscle-derived extracellular signal-regulated kinases 1 and 2 are required for the maintenance of adult Myofibers and their neuromuscular junctions. *Mol. Cell. Biol.*, **35**, 1238–1253.
 47. Bamshad, M., Van Heest, A.E. and Pleasure, D. (2009) Arthrogryposis: a review and update. *J. Bone Joint Surg. Am.*, **91**, 40–46.
 48. Wu, S.H., Arévalo, J.C., Sarti, F., Tessarollo, L., Gan, W.-B. and Chao, M.V. (2009) Ankyrin repeat-rich membrane spanning/Kidins220 protein regulates dendritic branching and spine stability in vivo. *Dev. Neurobiol.*, **69**, 547–557.
 49. BRIGHTcore. BRIGHTcore <http://www.brightcore.be> (accessed May 31, 2019).
 50. Li, H. and Durbin, R. (2009) Fast and accurate short read alignment with burrows-wheeler transform. *Bioinforma*, **25**, 1754–1760.
 51. Picard Tools - By Broad Institute. <https://broadinstitute.github.io/picard/> (accessed May 31, 2019).
 52. DePristo, M.A., Banks, E., Poplin, R., Garimella, K.V., Maguire, J.R., Hartl, C., Philippakis, A.A., del Angel, G., Rivas, M.A., Hanna, M. et al. (2011) A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat. Genet.*, **43**, 491–498.
 53. Highlander - home (Internet). <https://sites.uclouvain.be/highlander/documentation.html> (accessed May 31, 2019).
 54. Lek, M., Karczewski, K.J., Minikel, E.V., Samocha, K.E., Banks, E., Fennell, T., O'Donnell-Luria, A.H., Ware, J.S., Hill, A.J., Cummings, B.B. et al. (2016) Analysis of protein-coding genetic variation in 60,706 humans. *Nature*, **536**, 285–291.
 55. 1000 Genomes Project Consortium, Auton, A., Brooks, L.D., Durbin, R.M., Garrison, E.P., Kang, H.M., Korbel, J.O., Marchini, J.L., McCarthy, S., McVean, G.A. and Abecasis, G.R. (2015) A global reference for human genetic variation. *Nature*, **526**, 68–74.
 56. Exome Variant Server - Search Results. <https://evs.gs.washington.edu/EVS/PopStatsServlet?searchBy=Gene+Hugo&target=kidins220&x=0&y=0> (accessed Jun 4, 2019).
 57. Genome of the Netherlands. Genome of the Netherlands <http://www.nlgenome.nl/> (accessed June 4, 2019).
 58. Atherosclerosis Risk in Communities. Atherosclerosis risk in communities study description. https://sites.csc.unc.edu/atic/desc_pub (accessed June 4, 2019).
 59. Yates, A., Akanni, W., Amode, M.R., Barrell, D., Billis, K., Carvalho-Silva, D., Cummins, C., Clapham, P., Fitzgerald, S., Gil, L. et al. (2016) Ensembl 2016. *Nucleic Acids Res.*, **44**, D710–D716.
 60. Cingolani, P., Platts, A., Wang, L.L., Coon, M., Nguyen, T., Wang, L., Land, S.J., Lu, X. and Ruden, D.M. (2012) A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster* strain w1118; iso-2; iso-3. *Fly (Austin)*, **6**, 80–92.