ORIGINAL ARTICLE

Homozygous KIDINS220 loss-of-function variants in fetuses with cerebral ventriculomegaly and limb contractures

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Abstract

Heterozygous mutations in KIDINS220 were recently suggested a cause of spastic paraplegia, intellectual disability, nystagmus and obesity. All patients carried terminal nonsense de novo mutations that seemed to escape nonsense-mediated mRNA decay. The mechanism for pathogenicity is yet unexplained, as it seems that heterozygous loss-of-function variants of KIDINS220 are generally well tolerated. We present a consanguineous couple who experienced four pregnancy terminations due to repeated findings in the fetuses comprising enlarged cerebral ventricles and limb contractures. Exome sequencing in two of the aborted fetuses revealed a shared homozygous frameshift variant in exon 24 in KIDINS220. Sanger sequencing of the variant in available family members showed complete segregation with the affection status, resulting in a LOD score of 2.5 under an autozygous inheritance model. mRNA studies revealed destruction of the original splice site, resulting in an out-of-frame transcript and introduction of a premature termination codon in exon 25. Premature termination codons in this position are likely to cause activation of nonsense-mediated mRNA decay and result in complete absence of KIDINS220 protein in individuals homozygous for the variant. The phenotype of the presented fetuses overlaps with findings in functional studies of knockout Kidins220 mouse embryos that are non-viable with enlarged cerebral ventricles. The human fetuses also exhibit several similarities to the milder phenotype described in patients with heterozygous KIDINS220 mutations. We hence propose that the identified homozygous loss-of-function variant in KIDINS220 causes the phenotype in the presented fetuses, and that this represents a hitherto undescribed severe autosomal recessive neurodevelopmental disorder.

Introduction

The Kinase D-interacting substrate of 220kDa (KIDINS220) is a transmembrane scaffold protein which has recently been given much attention due to its complex, but crucial role in central nervous system (CNS) and cardiovascular system (CVS) development as demonstrated by functional studies in mice (1–3). Kidins220 knockout mice embryos show progressive dilatation of cerebral ventricles and die in late stages of gestation, seemingly from heart failure (1). Furthermore, heterozygous terminal loss-of-function (LoF) mutations in KIDINS220 were recently suggested as a cause of a recognizable syndrome in humans, comprising spastic paraplegia, intellectual disability, nystagmus and obesity (4). The identified mutations were all terminal, and Western blot analyses confirmed presence of truncated KIDINS220 protein in all the patients (4). As LoF mutations in KIDINS220 seem to be well tolerated, as demonstrated by the
observed number of LoF variants (n = 15) in healthy carriers in the ExAC database (5) (http://exac.broadinstitute.org), disease in heterozygotes may be caused by other means than haploinsufficiency. On the other hand, healthy individuals with homozygous LoF variants in this gene are not reported.

We present a consanguineous couple (first cousins) who experienced four provoked abortions due to severe and progressive dilatation of the cerebral ventricles, and extensive limb contractures observed on fetal ultrasound in week 18. A frameshift variant in exon 24 of the KIDINS220 gene segregated with disease in six family members where DNA was available. Homozygosity was shown in samples available from three affected fetuses, and healthy family members were either carriers or homozygous for the wild-type allele. As opposed to the variants found in the healthy family members, the single-point LOD score for the variant c.3394_3403del in exon 24 of KIDINS220 was further ruled out in their healthy child (exact genotype withheld). Assuming a first-cousin relationship between the parents, the variant was found to be technical artefacts. The variants in the remaining three genes were predicted to be benign, and the variants were found to be technical artefacts. The variants were hence considered unlikely to be involved in the disease.

In addition to the nine autozygous variants, 12 variants distributed over six genes were identified in a general recessive analysis (Supplementary Material, Table S4). In three of these, the variants were found to be technical artefacts. The variants in the remaining three genes were predicted to be benign, and they were hence considered unlikely to be involved in the disease. Finally, to examine for the possibility of dominant disease as a result from germline mosaicism in one of the parents, a search for rare, shared heterozygous variants predicted to be damaging in known disease genes was also performed. This did not yield any plausible candidate variants.

Sanger sequencing confirmed complete segregation of the KIDINS220 variant with disease in the family

Sanger sequencing of DNA from fetus F4 confirmed homozygosity for the variant c.3394_3403del in exon 24 of KIDINS220 (NM_020738) whereas the parents were heterozygous carriers. Homozygosity for the variant was further ruled out in their healthy child (exact genotype withheld). Assuming a first-cousin relationship between the parents, the single-point LOD score for the variant was computed to 2.5.

mRNA studies demonstrated aberrant splicing and introduction of a premature termination codon

The identified LoF variant was predicted to destruct the original splice site (Supplementary Material, Fig. S2). mRNA studies of the parents confirmed a destruction of the original splice site, moving the original splice site ten base pairs upstream. This results in an out-of-frame transcript and the introduction of a premature termination codon (p.Gln1132Serfs*30) (Fig. 1), which would induce NMD in any of the hitherto known splice isoforms found by Schmieg et al. (7).

Discussion

The KIDINS220 protein plays an important role in the developing brain and heart (3,7). Recent studies have shown how the protein is tightly regulated in fetal development with a variety

Table 1. Fetal ultrasound and autopsy findings in the four affected fetuses

<table>
<thead>
<tr>
<th>Individual (GA-weeks)</th>
<th>Fetus F1 (18–21)</th>
<th>Fetus F2* (18–19)</th>
<th>Fetus F3 (13)</th>
<th>Fetus F4 (18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limb contractures</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Hydrocephalus/dilated cerebral ventricles</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Small cerebellum</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Corpus callosum agenesis</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Comments</td>
<td>Low weight of the brain</td>
<td>Choroid plexus cyst</td>
<td>Thin cortex, collapsed brain</td>
<td>Micrognathia.</td>
</tr>
</tbody>
</table>

GA, gestational age; US, ultrasound; NK, not known.

aMedical record not available.
bInformation through medical history.
cCerebellum not identified.
dCerebellum not commented.

dCerebellum not commented.
of alternative splicing isoforms with highly specific spatiotemporal patterns of expression (1,3,7). So far, KIDINS220-related morbidity in humans has only been described in three patients with spastic paraplegia, intellectual disability, nystagmus and obesity, who all had a de novo heterozygous loss-of-function mutation within the last two exons of KIDINS220 (4). We present a consanguineous family with a seemingly severe neurodevelopmental disorder in four fetuses. In the three affected fetuses (F1, F3, F4) from whom DNA was available, we identified a homozygous frameshift deletion in exon 24 of KIDINS220 which co-segregated with disease under a recessive inheritance model. This yielded a single point LOD score of 2.5, i.e. close to the traditional significant threshold of 3.

The clinical findings of the fetuses in the present study were more severe than, yet overlapping with, the findings reported by Josifova and colleagues (4). Fetuses F1 and F4 showed severe and progressive dilatation of cerebral ventricles and characteristic limb contractures by week 18, whereas fetus F3 showed only mild contractures, but was aborted at an earlier gestational age (week 13). Fetus F4 further had agensis of the corpus callosum. Another pregnancy of the same couple was also terminated due to findings of enlarged cerebral ventricles, but no DNA was available from this fetus (F2). Of note, all three patients in the report of Josifova et al. exhibited pre- (from 20 weeks gestation) and postnatal cerebral ventriculomegaly, and one patient showed partial agenesis of the corpus callosum. Furthermore, all three patients had spastic paraplegia, and we speculate that the limb contractures observed in our fetuses present the more severe end of this phenotype.

In the ExAC population frequency database there are observed 15 different LoF variants in KIDINS220. This is lower than the expected number (58), but still results in a very low overall probability of loss-of-function intolerance (pLI = 0.03) (5), suggesting that certain LoF variants can be tolerated in heterozygous state. For instance, this may apply to variants causing premature termination codons subjected to NMD. The beneficial effect of NMD is well studied and limits the possibly toxic effects caused by a truncated protein (8). Moreover, there are no individuals with homozygous KIDINS220 LoF variants in databases of normal variation, suggesting that recessive LoF variants may not be tolerated. In Josifova’s report, all patients carried terminal nonsense variants and even the most proximal of these was shown to escape NMD, thus truncated forms of KIDINS220 protein were produced (4). The authors pointed to an imbalance in different isoforms of the KIDINS220 protein available for developing neurons as a possible mechanism for disease (4). The more proximal location of the premature termination codon (PTC) created by the frameshift variant described in this study, would be expected to result in NMD in all of the isoforms of KIDINS220 described so far (7). The localization of PTCs is crucial and has the potential to alter the inheritance from recessive to dominant, as can be seen in other syndromes (9), e.g. β-thalassemia (10) and Thomsen- and Becker-type myotonia (11). Due to the overlap in phenotype seen in heterozygous and homozygous patients, one may speculate that similar mechanisms come to play in the determination of dominant and recessive KIDINS220-related morbidity.

The major role of the KIDINS220 protein in several growth- and differentiation pathways in the nervous and cardiovascular system was demonstrated by Cesca and colleagues who showed that Kidins220 0/0 mice embryos die at late stages of gestation and show extensive neuronal cell death in the central and peripheral nervous systems (1,3). Abnormal cerebral ventricular dilatation was seen secondary to reduced brain mass, seemingly resulting from impaired differentiation and extensive cell loss (apoptosis) rather than impaired neuronal proliferation (3). The changes in the CNS seen in Kidins220 knockout mice further appeared from mid-gestation and progressed during the last stages of embryonic development, suggesting that Kidins220 is a promoter of neuronal survival from mid-gestation until birth (1). The observations in Kidins220 knockout mice fits with the findings in the fetuses presented here, where CNS changes were not seen on ultrasound until week 18 and seemed to be progressive during development. We suspect that the extensive limb contractures are secondary to loss of brain neurons as seen in mouse embryos, and that this process must have already started in fetus F3 before CNS changes were visible on ultrasound. The neurologic origin of the fetal limb contractures is further supported by normal findings on skeletal x-ray in fetus F1.

Kidins220 knockout mouse embryos further displayed heart malformations comprising dilated atria and histological changes of the ventricle wall and it was speculated that the cause of perinatal lethality in these embryos was heart failure (1). No heart malformations were shown in the three fetuses presented here, and microscopic investigations of fetal myocardium were also normal (investigated in fetus F4 only). The previously reported patients with heterozygous mutations in KIDINS220 did not show any heart anomalies either. One reason for the lack of heart pathology in the fetuses could be that the heart malformations become evident at a later gestational age than 18 weeks. Another explanation could be that the role of KIDINS220 in heart development differs between humans and mice. Although KIDINS220 is known to be expressed in heart in both species, the splicing patterns are not the same and the splicing diversity is known to be greater in mice (7).

A possible weakness of this study is that we can only make the assumption that the phenotypes in the four fetuses represent the same disease since pregnancy termination was carried out earlier in one of the pregnancies due to limb anomalies similar to the other affected fetuses. Hence we can only speculate that this fetus (F3) would also have developed dilated ventricles with time had the pregnancy carried on. Indeed the neuropathological investigation of this fetus (F3) showed a collapsed brain and seemingly thin cortex, which could imply that ventriculomegaly was beginning to develop. Furthermore, all three pregnancies from which DNA analyses have been performed were followed by the same obstetrician who considered the detected fetal abnormalities to be of the same nature, despite the lack of

**Figure 1.** Schematic illustration of the terminal exons of KIDINS220 mRNA. Grey colored exonic areas indicate regions where a premature termination codon (PTC) would be predicted to elicit nonsense-mediated mRNA decay (NMD), whereas the blue colored exonic areas would be predicted to escape NMD in the case of a PTC. A vertical line separates the two regions at position -155bp upstream of the last exon-exon junction. The c.3394_4034del; p.(Gln1132Serfs*30) deletion in exon 24 moves the splice site ten base pairs upstream of the original site. Furthermore, it disrupts the reading frame, leading to a PTC in exon 25, thus eliciting NMD. This is in contrast to the loss-of-function (LoF) variants reported by Josifova and colleagues, where all are predicted to escape NMD. "LoF variants reported by Josifova et al.: c.4050G>A; p.(Trp1350), c.4096C>T; p.(Gln1366) and c.4520dup; p.(Leu1507Phefs*4)."
ventriculomegaly in fetus F3. We also lack DNA from one fetus (F2) which based on medical history was also aborted due to hydrocephalus.

In addition to the KIDINS220 variant, the autozygosity mapping and segregation studies in the family resulted in one other homozygous loss-of-function variant segregating with disease, in the gene DSG4 (OMIM 607892). Homozygous and compound heterozygous variants (including nonsense variants) in DSG4 are known to cause non-syndromic hypotrichosis (Hypotrichosis 6, OMIM 607892). We therefore found this gene an unlikely causal candidate for the phenotype in this family.

In conclusion, we have found homozygosity for a loss-of-function KIDINS220 variant segregating with enlarged cerebral ventricles and limb contractures. The fetuses exhibit an overlapping phenotype of that seen in knockout Kidins220 mouse embryos and in three patients recently presented with terminal heterozygous mutations in KIDINS220 escaping NMD. We propose that the complete lack of KIDINS220 is the cause of the phenotype in the presented fetuses and hence represents a novel autosomal recessive neurodevelopmental disorder.

Materials and Methods

Clinical investigations and autopsies

Clinical features of the four fetuses are summarized in Table 1. Further details are supplied in Supplementary Material.

DNA sequencing and bioinformatic handling

DNA was obtained from fetus F1, F3 and F4. Exome sequencing of DNA from fetuses F1 and F3 was carried out applying Agilent SureSelect XT V5 kit (Agilent Technologies, Santa Clara, CA), and subsequent sequencing on Illumina HiSeq (Illumina, San Diego, CA, USA). Alignment to the human reference genome (hg19) was done with bwa-mem (version v0.7.12) (12). Joint variant calling on the two exomes was performed using the UnifiedGenotyper in the Genome Analysis Toolkit (GATK, v3.4) (13–15) and result- ing variants were annotated with Annovar (16).

Exome data analyses, Sanger sequencing of disease variant candidates and linkage analysis

Variant filtering and downstream analysis were performed in FILTUS (6). Identification of autozygous regions was done using the AutEx algorithm in FILTUS, after removing low quality variants (see Supplementary Material, Tables S1A and S1B for exact filters). Within regions shared by the two sequenced fetuses we listed all rare PASS variants (exact filters in Supplementary Material, Table S3). As secondary analyses, a general search for (simple or compound) recessive variants was done (exact filters in Supplementary Material, Table S4) as well as a dominant analysis looking for shared, rare variants predicted to be damaging (CADD > 20) in known disease genes. Identified disease variant candidates were confirmed with Sanger sequencing, and also tested in additional family members where DNA was available. For variants identified with autozygosity mapping, a single-point LOD score was computed with the R package paramlink (https://CRAN.R-project.org/package=paramlink), assuming a first-cousin relationship between the parents, and a fully penetrant recessive disease model with disease allele frequency of 0.00001.

DNA from fetus F4 was of too low concentration to perform high throughput sequencing. However, Sanger sequencing of the candidate variant was performed and confirmed homozygosity in fetus F4. Further confirmation of the variant was performed by Sanger sequencing in all family members where DNA was available, including the three fetuses, both parents and their healthy child. Additionally aCGH (180K) and karyotyping had previously been performed in DNA from fetus F1 in a diagnostic setting.

mRNA analyses

Total RNA was isolated from peripheral blood mononuclear cells from the parents carrying the variant and two healthy controls using the PAXGene Blood RNA kit (Qiagen, Valencia, CA, USA) according to the manufacturers’ recommendation. RNA concentration was assessed by Nanodrop1 ND-1000 UV–vis Spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA). Reverse transcription was performed with QIAGEN OneStep RT-PCR kit (Qiagen, Valencia, CA, USA) with 200 ng mRNA input and 15 μM of specific primers (5’-ATC CTT GTG CTC TTC CAC GT-3’, 5’- CCT CCT TGA TAA CTT CTA GGC CAT-3’) in a final volume of 24 μl. PCR products were purified and Sanger sequenced.

Study approval

The project was approved by the Norwegian Regional Ethical Committee, and the parents have given their written informed consent to the publishing of the findings.

Supplementary Material

Supplementary Material is available at HMG online.

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Conflict of Interest statement. None declared.

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References


