Short Report

NPHP4 mutation is linked to cerebello-oculo-renal syndrome and male infertility


Nephronophthisis is the most common genetic cause of renal failure in children and young adults. It is genetically heterogeneous and can be seen in isolation or in combination with other ciliopathy phenotypes. Here we report an index case where nephronophthisis is associated with oculomotor apraxia and cerebellar abnormalities, consistent with the clinical diagnosis of cerebello-oculo-renal syndrome. Prompted by a family history of an uncle with early onset end stage renal failure and infertility, we performed semen analysis on the index. This revealed marked reduction in the count of motile sperms as well as multiple abnormalities in the head and tail. Autozygome-guided mutation analysis followed by exome sequencing and segregation analysis revealed a homozygous truncating mutation in NPHP4, indicating that mutations of this gene can on rare occasions cause cerebello-oculo-renal syndrome. Our finding of severe male infertility in a family with NPHP4 truncation is strongly supported by the mouse model and, to our knowledge, is the first reported male infertility phenotype in association with NPHP4 or any other nephrocystin in humans.

Conflict of interest

The authors declare no conflict of interest.

Nephronophthisis is a renal disease characterized clinically by polydipsia, polyuria and progressive renal failure (1). Although only 5% of pediatric end stage renal failure (ESRF) is attributed to nephronophthisis, this condition is the most common genetic cause of ESRF in pediatrics and young adults (2, 3). Cyst formation at the cortical–medullary junction and renal fibrosis are characteristic pathological features which can also be detected by ultrasonography (3). The only known mode of inheritance in this condition is autosomal recessive, and 15 loci have been mapped to date. All disease genes appear to encode proteins that are related to the cilia, a microtubule-based cellular appendage with an increasingly recognized role in signaling and cell polarity (4).

NPHP4 encodes nephrocystin-4, a highly conserved protein that is necessary for the proper formation of...
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the primary cilium but which has also been linked to cell–cell adhesion and proliferation (5). Mutations in this gene account for less than 3% of all cases of nephronophthisis and while most of these appear to be isolated, other phenotypes related to disordered ciliary biology (collectively known as ciliopathies) have uncommonly been reported, most notably retinal degeneration (known as Senior-Loken syndrome) (6).

In this study, we report a highly unusual case of nephronophthisis that additionally displays cerebellar involvement and oculomotor apraxia thus fulfilling the clinical definition of cerebello-oculo-renal syndrome. In addition to representing the first case of cerebello-oculo-renal syndrome caused by NPHP4 mutation, we demonstrate for the first time human male infertility in patients with a homozygous truncating mutation in this gene. This study, therefore, significantly expands the phenotypic consequences of NPHP4 mutations in humans although the nature of phenotype/genotype correlation, if any, remains unclear.

Subjects and methods

Human subjects

The index was recruited under a KFSHRC IRB-approved research protocol (RAC #2080006) with written informed consent. Full clinical evaluation included a detailed family history, neurologic and clinical genetic assessment, imaging studies on the brain and kidneys, and semen analysis. Blood was drawn in ethylenediaminetetraacetic acid (EDTA) from the index and available family members for DNA extraction and downstream molecular studies.

Autozygome analysis and exome sequencing

This was essentially as described before (7). Briefly, the Affymetrix Axiom genotyping platform generated high-density single-nucleotide polymorphism (SNP) genotypes which were then analyzed by AutoSNPa to impute runs of homozygosity that were greater than 2 Mb in size. These were used as surrogates of autozygosity, and the entire set of such runs was used to construct the autozygome. When known cerebello-oculo-renal syndrome genes within the autozygome were excluded, exome sequencing was performed as described before. Once the causative mutation was found, the presence of heterozygous truncating or damaging mutations was excluded from the following list of known ciliopathy genes with autosomal recessive aging mutations was excluded from the following list:

- INPP5E
- PKHD1
- TCTN1
- MKKS
- DNAH5
- CEP41
- BBS12
- TTC8
- B9D1
- TTD1
- DNAH8
- INTU
- CEP152
- RSPH4A
- RSPH9
- SDCCAG8
- TCTN1
- TCTN2
- TMEM67
- TMEM138
- TMEM216
- TMEM231
- TMEM237
- TRIM32
- TTC21A
- TTC21B
- TTC8
- TXNDC3
- WDR55
- XPNPEP3
- ZNF423

No known cerebello-oculo-renal syndrome disease genes were found to reside within the autozygome of this patient. Subsequent exome sequencing uncovered 82,161 variants; these were filtered to include only homozygous alterations given the presence of consanguinity. After confirming that there were no pathogenic variants in any of the known cerebello-oculo-renal

Results

Clinical report

The index is a 17-year-old Saudi male who was referred for clinical genetics evaluation because of chronic renal disease, abnormal eye movement and mild intellectual disability.

His pregnancy and delivery were unremarkable. Nystagmus and inability to track with his eyes were noticed since early infancy. In addition, he was noticed as an infant to be floppy and to have delayed milestones. Although he attends regular school, he has a very poor academic performance but no formal intelligence quotient (IQ) testing was performed. Abnormal renal function was evident by 3 years of age but has progressed very slowly since then. He was diagnosed by his nephrologist as suffering from chronic kidney disease. The initial evaluation at age 14 revealed a urea level of 20 mmol/l and creatinine level of 264 μmmol/l. Now (3 years later) the urea level is 14.1 mmol/l and creatinine is 333 μmmol/l (the upper limits are 6 and 115, respectively). His parents are first degree Saudi cousins and his paternal uncle is a 45-year-old male with nystagmus, early onset ESRF and long standing infertility (Fig. 1a). His facial profile is similar to the proband and he did not complete his schooling.

His physical examination was significant for a thin teenager, his growth parameters were: weight 41 kg (~2.8 SD), height 166 cm (11th percentile) and head circumference 54.5 cm (34th percentile). He had a triangular face, thick eye brows, prominent nasal bridge, and broad nasal tip (Fig. 1b). There was severe inability to track with his eyes (apraxia) with occasional nystagmus. Retinal examination revealed no major abnormalities. Neurological examination was notable for mild exaggeration of deep tend reflexes in the extremities and his higher cognitive functions were below average.

Brain MRI showed thickening of the superior cerebellar peduncle and dysgenesis of the corpus callosum involving its splenium (Fig. 1c). The molar tooth sign, a common but non-essential component of classical Joubert syndrome (8), was not observed. Renal ultrasonography revealed bilaterally small kidneys with increased echogenicity and cystic dysplasia (Fig. 1d). Semen analysis revealed a highly viscous, low volume sample with low total motile sperm count of 11 million (normal >40 million) and significant abnormalities in the sperm’s morphology (seen in 90% of the sperm’s) (Fig. 2). For the paternal uncle, total sperm count was 9 million/ml (normal value is 19 million/ml).

Identification of a homozygous truncating mutation in NPHP4

No known cerebello-oculo-renal syndrome disease genes were found to reside within the autozygome of this patient. Subsequent exome sequencing uncovered 82,161 variants; these were filtered to include only homozygous alterations given the presence of consanguinity. After confirming that there were no pathogenic variants in any of the known cerebello-oculo-renal...
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syndrome disease genes genomewide, we expanded our search to encompass all genes within autozygous regions >1 Mb (Fig. 1e), and uncovered a homozygous truncating mutation in NPHP4 (c.2044C>T, p.R682*; NM_015102.3) (Fig. 1f). Interestingly, the uncle was also found to be homozygous for this mutation which was once reported in the heterozygous state in a patient with isolated nephronophthisis (9).

To assess whether a second recessive disorder was causing the infertility, we examined all homozygous coding and splice site variants which were present in our exome data in regions of shared autozygosity between the proband and his affected uncle. Our search included all coding and splice site SNPs with low (<1%) population frequencies. Variants were interrogated against a database of 250 ethnically matched exomes. In the end, the only variant remaining was the stopgain mutation in NPHP4. To make our genetic assessment more comprehensive, we also searched for truncating or damaging heterozygous mutations in known ciliopathy genes that undergo autosomal recessive inheritance (these are listed in the “Subject and methods” section). We did not find suspicious variants in any of the genes tested, further implicating NPHP in the disease pathogenesis in this family.

Discussion

Allelism, or the capacity of mutations within the same gene to cause different clinical phenotypes, is well established in ciliopathies and nephronophthisis. However, no exception. Indeed, the entire spectrum ranging from isolated nephronophthisis to the perinatal lethal Meckel–Gruber syndrome has been observed in connection with mutations in the various nephronophthisis disease genes (3, 4, 6). The exact mechanism for this phenomenon remains unclear and is made more complicated by the observation that sometimes the same mutation can result in isolated nephronophthisis or a more complex ciliopathy phenotype. Modifying effects by other genes (cilia-related or otherwise) or even stochastic developmental events are likely to play a role.

NPHP4, like other nephronophthisis disease genes identified to data, encodes a protein that plays a critical role in ciliary function and structure (9, 10).
Knockdown of its expression is associated with abnormal ciliogenesis, and nephrocystin-4 has been shown to play a role in the proper localization of key ciliary proteins (5, 11). Although numerous NPHP4 mutations have been identified, several attempts have failed to find a consistent correlation between the mutation type and the resulting phenotype that ranges from isolated nephronophthisis to Senior-Loken syndrome to Cogan syndrome (nephronophthisis and oculomotor apraxia) (6, 12). Our finding of an NPHP4 mutation in a patient with cerebellar involvement further expands the spectrum of associated ciliopathies. RPGRIP1L, a known Joubert disease protein, interacts in vitro and in vivo with nephrocystin-4 which provides a hint towards the likely mechanism between the association we describe here (13). Of note, the homozygous truncating mutation we report here has previously been reported in the heterozygous state in a patient with isolated nephronophthisis (6, 9). Since only biallelic mutations are known to cause nephronophthisis, those authors speculated that the other allele may have been missed or that the mutation may represent a sequence variant not related to the phenotype. Thus, our finding supports the pathogenic nature of this mutation in the heterozygous state as a likely cause of a syndromic form of nephronophthisis.

Interestingly, a naturally occurring truncating mutation in NPHP4 has been reported in dogs but the phenotype is restricted to the retina (14). It was proposed that the preserved N-terminus of the truncated NPHP4 allows for the interaction with nephrocystin to remain intact thus preventing renal involvement. However, an N-ethyl-N-nitrosourea (ENU)-induced truncating mutation in the mouse ortholog of NPHP4 was found to completely destabilize the protein and yet the renal involvement was curiously lacking (15). Thus, it appears that the preservation of the binding domain to other nephrocystins is not necessary to prevent nephronophthisis, at least in mouse. Nevertheless, that mouse model, in addition to recapitulating the retinal phenotype seen in 10% of nephronophthisis patients, displayed an unexpected reproductive phenotype that has hitherto been unknown in humans (15). Male mice were infertile and had marked reduction in sperm count. Importantly, sperms from these mice failed to fertilize eggs in vitro confirming that the cause of infertility is not limited to the reduced count but extends to abnormal sperm physiology as well. Indeed, in the family we report, we were able to document markedly abnormal sperm morphology. To our knowledge, this is the first reported male infertility phenotype in association with NPHP4 or any other nephrocystin in humans. As in the mouse model, the exact mechanism remains unclear but it is worth mentioning that Nphp1 −/− mice also display male infertility (16).

In summary, we report a family with homozygous NPHP4 truncating mutation that expands the phenotypic spectrum of NPHP4-related nephronophthisis to also include cerebello-oculo-renal syndrome and abnormal spermatogenesis causing male infertility. We suggest that NPHP4 should be screened in the mutation analysis of cerebello-oculo-renal syndrome patients and that further studies are warranted regarding male fertility in patients with nephronophthisis to fully explore the causal link we propose in this study.

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