SHORT REPORT

Mutations in TMEM231 cause Meckel–Gruber syndrome

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ABSTRACT

Background Meckel–Gruber syndrome (MKS) is a genetically heterogeneous severe ciliopathy characterised by early lethality, occipital encephalocele, polydactyly, and polycystic kidney disease.

Purpose To report genetic analysis results in two families in which all known MKS diseases genes have been excluded.

Methods In two consanguineous families with classical MKS in which autozygome-guided sequencing of previously reported MKS genes was negative, we performed exome sequencing followed by autozygome filtration.

Results We identified one novel splicing mutation in TMEM231, which led to complete degradation of the mutant transcript in one family, and a novel missense mutation in the other, both in the homozygous state.

Conclusions TMEM231 represents a novel MKS locus. The very recent identification of TMEM231 mutations in Joubert syndrome supports the growing appreciation of the overlap in the molecular pathogenesis between these two ciliopathies.

BRIEF REPORT

Ciliopathies are multiorgan system disorders caused by dysfunction of the primary ciliary, a ciliary appendage which plays essential roles in cellular homeostasis and organ development.1 2 Despite the common defective ciliomplex, ciliopathies present diverse clinical features in addition to some overlapping phenotypes.3 4 The mechanism underlying the discordant clinical presentation of various ciliopathies has been poorly understood. Encouragingly, new revelations about cellular and developmental functions of ciliopathy associated genes have established an expanding network linking ciliopathies, cilium genes and their involvements in organ development, which provided an important stepping stone towards a better understanding of the genotype–phenotype correlation in these disorders.4 5 Meckel–Gruber syndrome (MKS) represents the severe end of the ciliopathy phenotypic spectrum. The disease is usually lethal shortly after birth, and affected children typically present with the triad of occipital encephalocele, polydactyly, and polycystic kidney disease, among other associated features.6 Twelve genes are known to cause this disorder when mutated: MKS1, TMEM216 (MKS2), TMEM231 (MKS3), CEP290 (MKS4), RPGRIP1L (MKS5), CC2D2A (MKS6), NPHP3 (MKS7), TCTN2 (MKS8), B9D1 (MKS9), B9D2 (MKS10), TMEM237, and C5orf42. Recently, we have suggested that mutations in EV22 and EXOC4 may also cause MKS.7 In this study, we show that TMEM231 is the latest gene to be linked to MKS pathogenesis based on pathogenic mutations we identified in two families.

Family 1 consists of first cousin parents who lost two pregnancies because of MKS diagnosed prenatally; one was spontaneously aborted while the other was terminated (figure 1). The current pregnancy was complicated by oligohydramnios and was highly suspicious for MKS because of occipital encephalocele, polydactyly, and polycystic kidney. Therapeutic abortion was performed at the request of the parents. The family was enrolled under KFSHRC IRB approved protocol KFSHRC RAC#2080006 with written informed consent, and blood samples were collected from both parents and from the fetus before abortion. DNA was extracted followed by autozygome analysis essentially as described before.8 The only known MKS gene that was found to reside within the autozygome of the affected child was excluded by sequencing, so we proceeded with exome sequencing. Only one homozygous variant in TMEM231 (NM_001077416.1: c.751G>A) survived the filtration scheme shown in figure 1B. This variant affects the last nucleotide in exon 4 so we predicted that it affects the adjacent donor site. Unfortunately, this splicing variant could not be experimentally verified in the index because of the unavailability of RNA, so we tested blood-derived RNA collected in PAXGene tubes from the parents. No aberrant band was apparent in the parents on reverse transcriptase PCR (RT-PCR) using TMEM231 cDNA primers (figure 1C). However, upon sequencing the resulting amplicon, there was another aberrant variant at the level of RNA, suggesting complete nonsense mediated decay (NMD), most likely triggered by aberrant splicing and introduction of a premature stop codon. In order to investigate the nature of the aberrant transcript directly, we established lymphoblastoid cell lines from both parents. In contrast to RNA that was directly extracted from blood, RNA derived from the cell lines contained aberrant TMEM231 transcript in low abundance, as indicated by a faint smaller band on RT-PCR using the same primers as in the first RT-PCR experiment on blood-derived RNA (see online supplementary figure S1). Cloning experiments revealed that it in fact represents two aberrant transcripts, one in which the splicing mutation resulted in the retention of 47 bp from intron 4 (10% of colonies), and another that retained 11 bp from the same intron (5% of colonies) (the remaining 85% of colonies contained a normal

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transcript from the wild type allele). Both aberrant transcripts predict frameshift and premature truncation (p.Val251Serfs*21 and p.Val251Serfs*9) so they are the likely trigger of NMD we observed in blood.

Interestingly, we observed a shared autozygous interval spanning 5.08 Mb between our patient and another patient we described in our recent MKS case series as the only case in which exome sequencing failed to identify a causal mutation after applying the various filters (case MKS_F18 in Shaheen et al)\textsuperscript{7} (figure 1D). Re-examination of the exome variants from MKS_F18 in this interval revealed a heterozygous missense variant in \textit{TMEM231} (NM_001077416.1:c.902A>C) that had not been considered because it was heterozygous. However, Sanger sequencing showed that this variant is in fact homozygous, suggesting a technical error in the calling of this variant during exome sequencing. The affected residue is absolutely conserved across species (figure 1E) and is highly predicted to be pathogenic based on PolyPhen-2 and SIFT (Sorting Intolerant From Tolerant). Both homozygous variants are pathogenic and likely to be disease-causing.

\textit{TMEM231} was recently identified as a protein that forms a complex with B9 and CC2D2A—two established MKS genes—at the transition zone of cilia, and that its deficiency at the cellular and organism level leads to ciliopathy.\textsuperscript{9} Very recently, compound heterozygosity for two mutations in \textit{TMEM231} was identified in three patients with Joubert syndrome.\textsuperscript{10} All three patients met the classical definition of Joubert syndrome as they had the constellation of oculomotor apraxia, molar tooth sign on brain MRI, breathing abnormalities, and developmental delay. While a subset of Joubert patients may have polydactyly and renal involvement like MKS, the clinical distinction between the two disorders is usually apparent. Thus, our finding of two pathogenic mutations in patients with classical MKS expands the list of genes that cause both disorders to also include \textit{TMEM231}. Similarly, as we have shown for \textit{TMEM237} and \textit{C5orf42}, the correlation between the genotype of \textit{TMEM231} mutations and the resulting phenotype being Joubert syndrome or MKS remains undefined. It is unclear why the complete loss of function and missense mutations in \textit{TMEM231} found in our patients caused classical MKS while the compound heterozygous mutations in the same gene found in Srour’s report resulted in Joubert syndrome.\textsuperscript{10} The consistent phenotype within a given family argues against stochastic factors during development. More likely, it appears that the phenotype is either allele-specific or at least genetic background-specific within a given family. Clearly, this will be the subject of future investigation once the cost of sequencing drops to a level that allows massive sequencing of entire cohorts of ciliopathy patients to assess for the contribution of modifiers.
In summary, we report the first MKS patients with TMEM231 mutations. This study, combined with the recent identification of TMEM231 mutations in Joubert syndrome, confirm the designation of TMEM231 as a ciliopathy gene with variable phenotypic consequences.

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Competing interests None.

Patient consent Obtained.

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