***TMEM67* mutations found in a case of Joubert syndrome with renal hypodysplasia**

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Received: 27 October 2015 / Accepted: 21 December 2015 / Published online: 21 January 2016
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Abstract Joubert syndrome is a rare inherited cerebellar ataxia with the dysgenesis of the cerebellar vermis, called the molar tooth sign. The combination of a large number of causative genes, more than 27, and the various clinical features involving multiple organs has established many genotypic-phenotypic correlations in Joubert syndrome. *TMEM67* is one of the genes that are relatively well established as contributing to Joubert syndrome with liver involvement. Here, we report a 2-month-old boy who was initially treated for urinary tract infection, which further led to the diagnosis of Joubert syndrome accompanied by renal hypodysplasia with two different mutations: c.2522A>C and c.1065 + 4Adel in *TMEM67*.

Keywords Joubert syndrome · Molar tooth sign · Renal hypodysplasia · Whole-exome sequencing

Introduction

Joubert syndrome (MIM213300) is a rare inherited cerebellar ataxia, first described in a family with the dysgenesis of the cerebellar vermis [1]. Although the epidemiological data for Joubert syndrome are scarce, its prevalence is suggested to be between 1 per 80,000 and 1 per 100,000 live births [2]. Joubert syndrome was characterized by the

abnormal cerebellum and brainstem, called the molar tooth sign [3]. However, the accumulated cases diagnosed based on the molar tooth sign by brain imaging revealed the remarkable complexity of the clinical pictures of Joubert syndrome. Those patients manifested with multiorgan involvement, including the orofacial region, retina, liver and kidney, engendering the term “Joubert syndrome and related disorders” [2, 4]. However, such extreme phenotypic variability and emerging genetic complexity have caused much confusion in the classification of this disorder. For this reason, the diagnosis of these disorders is now proposed to revert from “Joubert syndrome” to all molar tooth sign-related disorders [2]. Therefore, the present report conveniently employs this classic term. We report a case of an infant boy who initially presented with urinary tract infection, which later led to the diagnosis of Joubert syndrome accompanied by renal hypodysplasia with two different mutations in the *TMEM67* gene.

Case report

A 2-month-old boy was admitted to Kyorin University Hospital due to fever of unknown cause. He was born at 36 weeks gestation by normal vaginal delivery and had a birth weight of 2692 g. His familial history was negative for neurological and renal diseases. Physical examination revealed ocular hypertelorism, bilateral ptosis and muscular hypotonia, but no findings indicating infectious diseases, including skin rash, neck stiffness, hepatosplenomegaly and lymph node swelling, were observed. Laboratory tests showed leukocyturia, bacteriuria (*E. coli*, 1×10^4 /ml) and raised levels of inflammatory markers in the peripheral blood (white blood cells, 15,000/ μ L). Blood examination indicated an absence of renal

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dysfunction and acidosis. He was diagnosed with upper urinary tract infection. High fever and inflammatory markers in the urine and blood were normalized 3 days after the initiation of intravenous antibiotic therapy with cefotaxime. A renal ultrasound of the right kidney revealed a highly echogenic organ containing small cysts without apparent corticomedullary differentiation (41×20 mm) (Fig. 1a). The left kidney (59×36 mm) was highly echogenic in the cortical region, like the right kidney, but no cysts were found (Fig. 1b). Voiding cystography revealed absence of vesicoureteral reflux. A Tc-MAG3 renogram showed a nonfunctioning pattern of the right kidney, while the left kidney appeared to function normally (Fig. 1e, f). At the age of 10 months, the delay of his neurological development became apparent; he had not achieved full head control yet. Thus, he underwent brain magnetic resonance imaging to identify a cause of the abnormal neurological delay. The scan revealed a small

vermis with abnormal vermian folia, dysplastic cerebellar hemispheres opposed in the midline and enlarged superior cerebellar peduncles with an absence of decussation, which represented the molar tooth sign (Fig. 2). Therefore, the clinical diagnosis of Joubert syndrome was eventually made. At the age of 2 years, his developmental delay was more apparent; he was unable to walk or sit up in bed because of cerebellar ataxia and muscular hypotonia. Hematology and biochemistry, including renal function (Cr, 0.3 mg/dL; cystatin c, 0.98 mg/L; HCO_3^- , 22.0 mEq/L) and liver enzymes, were normal. Urinary findings including β 2-microglobulin (341 $\mu\text{g/L}$) were normal. A renal ultrasound of the right kidney revealed it was similar in size as 2 years before (41×11 mm, Fig. 1c). The left kidney had an apparently extended size (74×42 mm); however, the cortical region was still highly echogenic, and one cyst of 6 mm diameter was found (Fig. 1d). Liver ultrasound revealed no abnormal finding at this moment.

Fig. 1 Renal morphology and function. Arrow indicates at the age of 2-months, a renal ultrasound revealed a highly echogenic organ containing small cysts without corticomedullary differentiation (41×20 mm) of the right kidney (a) and highly echogenic cortex, but without cysts of the left kidney (59×36 mm, b). At the age of 2 years, a renal ultrasound of the right kidney revealed similar size as 2 years before (41×11 mm, c). The left kidney had an apparently extended size (74×42 mm); however, the cortical region was still highly echogenic, and one cyst of 6 mm diameter was found (d). At the age of 3-months, Tc-MAG3 renogram showed a nonfunctioning pattern of the right kidney, while the left kidney appeared to function normally (e, f)

Fig. 2 T1-weighted brain magnetic resonance imaging at the age of 9 months revealed a typical molar tooth sign (arrow)

The patient has not revealed a relapse of the urinary tract infection until now.

Methods and results

Genomic DNA from peripheral leukocytes was used for whole-exome sequencing (WES) analysis after obtaining informed consent from the boy's parents. Genomic DNA was captured using the SureSelect Human All Exon v5 (50 Mb) Kit (Agilent Technologies, Santa Clara, CA, USA) and sequenced with a HiSeq 2000 (Illumina, San Diego, CA, USA) with 101 bp paired-end reads and 7 bp index reads. Exome data were used for mapping, variant calling, and variant annotation as previously described [5]. We found two mutations [c.2522A>C (p.Gln841Pro) and c.1065 + 4Adel] in *TMEM67* related to Joubert syndrome (Fig. 3). The two mutations were confirmed by Sanger sequencing, but we did not confirm their parental origin, as the parents refused the testing. However, we believe that the two mutations are on the different parental alleles, therefore fitting to the autosomal recessive model.

Discussion

Joubert syndrome is a recessively inherited disorder caused by any of 27 or more causative genes [6]. Compound heterozygote mutations of *TMEM67* were identified as causative in the present case. Mutations of *TMEM67*, also named *MKS3/NPHP11/JBTS6*, account for 6–10 % of cases of Joubert syndrome [2]. This gene was first identified as a causative gene of Meckel syndrome, a lethal

Fig. 3 Electropherograms of the two *TMEM67* mutations. A missense mutation (left): c.2522A>C (p.Gln841Pro) in exon 22 and a splice-donor site mutation: c.1065 + 4Adel, in intron 10

disorder displaying multicystic kidneys, CNS malformation, postaxial polydactyly and hepatic abnormalities, including portal fibrosis or ductal proliferation [7]. However, the prevalence of mutations of *TMEM67* in Meckel syndrome patients is actually not high, only 12–16 % [8]. Interestingly, Meckel syndrome shares 13 causative genes, including *TMEM67*, with Joubert syndrome, which might explain why there is striking clinical overlap between both disorders [7].

The combination of a large number of causative genes and the various clinical features involving multiple organs clearly establish the genotypic-phenotypic correlations of Joubert syndrome. *TMEM67* is one of the genes whose genotype-phenotype correlation is relatively well established in Joubert syndrome. Clinically, Joubert syndrome is categorized into several subgroups depending on the involvement of the retina, liver and kidney [2], in which congenital liver disease is believed to be strongly associated with *TMEM67* mutations. Indeed, *TMEM67* mutations account for approximately 80 % of Joubert syndrome patients with liver involvement, and congenital liver fibrosis is a main feature [2, 5]. Although our patient, currently at 2 years old, displays no liver damage as assessed by blood chemical tests and liver ultrasound, further continuous follow-up is needed to completely exclude liver involvement.

Mutations of *NPHP1*, *RPGRIP1L*, *CEP290*, *ZNF423*, *TMEM231* and *TMEM237* are associated with Joubert syndrome with kidney involvement, in which the major clinical picture consists of nephronophthisis and autosomal-recessive polycystic kidney disease [2]. In contrast, our patient had hypoplasia of the right kidney and highly echogenic areas with a small cyst on the left kidney, which suggests that the kidney involvement in the present patient is most likely renal hypodysplasia. Although a histological analysis of the

kidney is needed for accurate diagnosis in this case, a prominent unilateral kidney involvement is intriguing as a renal phenotype of Joubert syndrome. The splicing mutation identified in our patient has never been registered in the Human Genetic Variation Database (<http://www.genome.med.kyoto-u.ac.jp/SnpDB/index.html>) or the NHLBI Exome Sequencing Project (ESP6500). p.Gln841Pro, evaluated as damaging by PolyPhen2 (<http://genetics.bwh.harvard.edu/pph2/>), was reported in a Joubert syndrome patient with congenital hepatic fibrosis (COACH syndrome: MIM:216360) [9], which usually reveals nephronophthisis as the kidney involvement [4]. The pathogenic impact of p.Gln841Pro could be explained by the glutamine at position 841 of the protein product: meckelin is completely conserved among human, chimpanzee, dog, mouse, and rat. This amino acid is located at the cytoplasmic domain of meckelin. The cytoplasmic domain of meckelin interacts with the actin-binding protein filamin A, which potentially plays a crucial role in adequate cilium formation. Meckelin is expressed in the proximal renal tubules [10], and therefore, this mutation may lead to abnormal meckelin that is not able to interact with the cytoskeleton network in the proximal tubules. In addition, we found the splicing mutation c.1065 + 4Ade1. This mutation is speculated to lead to aberrant splicing, based on web-based programs: Berkeley Drosophila Genome Project (http://www.fruitfly.org/seq_tools/splice.html), ESEFinder (<http://rulai.cshl.edu/cgi-bin/tools/ESE3/eefinder.cgi>), and NetGene2 (<http://www.cbs.dtu.dk/services/NetGene2/>). Taken together, our results suggest that the protein product of this mutated *TMEM67* may interfere with the proper development of the proximal tubular cells because of ciliary dysfunction, leading to renal hypodysplasia. The significance of the unilaterality of the kidney involvement in our case requires further precise studies of the genotypic-renal phenotypic correlation in the *TMEM67* gene.

Acknowledgments The authors thank Dr. Yugo Ito and Noriko Ito-Nitta for the clinical management of the patient.

Compliance with ethical standards

Conflict of interest None.

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