A Homozygous Missense Variant in *INPP5E* Associated with Joubert Syndrome and Related Disorders

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Established Facts

- Mutations in the *INPP5E* gene are known to be associated with pure Joubert syndrome (JS) and Joubert syndrome and related disorders (JSRD) with variable features including retinal dystrophy, cystic echogenic kidneys, and hepatic disease.

Novel Insights

- A novel homozygous mutation at c.1303C>T (p.Arg435Trp) in the *INPP5E* gene is associated with JSRD.
- Our patient presented with clinical features of JSRD with nephropathy and no signs of retinopathy. This clinical variability can be due to different mutations leading to the formation of different proteins and thus exhibiting the different distortions of hydrogen bonds in the tertiary structure of proteins.

Keywords

Ciliopathy · *INPP5E* · JSRD

Abstract

Joubert syndrome and related disorders (JSRD; ORPHA 140874) is a complex set of neurodevelopmental disorders with multiple organ involvement. JSRD is a type of ciliopathy which is caused by the presence of defective primary cilia in an individual. JSRD is commonly inherited in an autosomal recessive pattern, and more than 23 genes are known to be

associated with JSRD. We report a novel homozygous mutation identified in the *INPP5E* gene, c.1303C>T, which leads to a change of an amino acid from arginine to tryptophan at residue 435 in the protein chain. In silico analysis indicates that p.Arg435Trp substitution affects the functionality of the protein product of the gene. Our result adds to the growing body of evidences that underlines the clinical utility of next-generation sequencing in the diagnosis of a genetic disorder when clinical features are inconclusive.

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Joubert syndrome (JS) is a multigenic and a heterogeneous class of ciliopathy which mainly affects the central nervous system, leading to hypotonia, ataxia, intellectual disability, developmental delay, impaired eye movement, and neonatal breathing difficulties [Parisi et al., 2007; Parisi, 2009]. The diagnosis is confirmed by the characteristic “molar tooth sign” (MTS) on MRI, which is characterized by elongated, thickened, and horizontally oriented superior cerebellar peduncles and deepened interpeduncular fossa [Choh et al., 2009; Doherty, 2009]. It is a genetic disorder inherited mostly in an autosomal recessive fashion and in X-linked manner in rare cases. There are 6 phenotypic subtypes of JS: pure JS, JS with ocular defects, JS with renal defects, JS with oculorenal defects, JS with hepatic defects, and JS with orofaciocdigital defects [Brancati, 2010]. When the characteristic features of JS occur in combination with one or more systems, the condition is referred as Joubert syndrome and related disorders (JSRD). To date, more than 23 genes are known to be associated with JSRD (*INPP5E*, *TMEM216*, *AHI1*, *NPHP1*, *CEP290* (*NPHP6*), *TMEM67* (*MKS3*), *RPGRIPL1*, *ARL13B*, *CC2D2A*, *OFD1*, *TTC21B*, *KIF7*, *TCTN1*, *TCTN2*, *TMEM237*, *CEP41*, *TMEM138*, *C5orf42*, *TCTN3*, *ZNF423*, *TMEM231*, *CSPP1*, and *PDE6D*) [Ben-Salem et al., 2014]. All these genes code for the protein of primary cilium. Cilia are hair-like structures or organelles which extend from the surface of nearly all cells and play a vital role in the development, functioning, and signaling of several cell types including retinal photoreceptors, neurons, kidney tubules, and bile ducts [Badano et al., 2006; Lancaster and Gleeson, 2009]. The organelles regulate via phosphatidylinositol signaling pathway [Waters and Beales, 2011; Basten and Giles, 2013]. Various homozygous and compound heterozygous mutations in the *INPP5E* gene have been reported responsible for JSRD with predominantly retinopathy; in some cases, cystic echogenic kidneys and hepatic fibrosis have also been reported [Parisi, 2009; Ben-Salem et al., 2014]. These mutations cause premature destabilization of cilia in response to stimulation. Recent advances in next-generation sequencing (NGS) technologies have revolutionized our ability to elucidate the genetic disorders. In this study, we report a case of a 6-year-old girl with a novel mutation c.1303C>T in *INPP5E*.

Patient and Methods

Clinical Report

The proband is a 6-year-old girl, referred to our department, who presented with generalized hypotonia, ataxia, speech delay,

low-set ears, ptosis, strabismus, clinodactyly, and a history of psychomotor developmental delay. She was born full term to a consanguineous couple by caesarean section with a birth weight of 2.26 kg. She was unable to hold her neck, sit, or crawl until 1.5 years and walked at about 3 years of age. At the age of 6 years, her head circumference was at the 50th percentile. Due to monosyllabic speech, she was undergoing speech therapy. Subsequently, a brain MRI showed MTS, and abdominal ultrasound revealed grade I nephropathy with medullary sponge kidneys. At the first visit, the proband's mother was pregnant with a gestation of 12 weeks, and the couple was worried about the risk of recurrence. They were informed about the importance of performing genetic investigation to achieve a correct diagnosis in order to determine the recurrence risk. Nuchal translucency (NT) scan was advised for the proband's mother, who showed abnormal posterior fossa with increased NT. Antenatally, abnormal posterior fossa is defined as one of the markers for JS/JSRD [Doherty et al., 2005]. In view of the abnormal NT and the possibility of recurrence, the couple decided to terminate the pregnancy. As the diagnosis for the proband was not established, NGS-based clinical exome sequencing was offered.

Methods

Clinical exome sequencing was performed targeting approximately 5,000 genes known to be involved in inherited disorders. High-quality genomic DNA was used to enrich the regions of interest using the SureSelect XT™ (Agilent Technologies, Santa Clara, CA, USA) in conjunction with the SureSelect Focused Exome™ kit. NGS was performed using the IlluminaNextSeq 500™ technology. The average exome-sequence coverage per nucleotide was 86× across the 12 Mb target. The quality control passed raw data were aligned to the human reference genome hg19. Only high-quality data (>Q30) were used for variant calling. Variant calling was performed using the GATK toolkit producing VCF file. The mapping and variant calling were executed on the BWA Enrichment App of Illumina BaseSpace. The VCF file was annotated by Variant Studio in Illumina BaseSpace for primary annotation. The partially annotated VCF file was then annotated for variant frequency in the EXAC database and 1000 Genome database using DRUVA 3.0 (in-house custom annotation pipeline of Dhiti Omics Technologies Pvt. Ltd., Bangalore). Finally, significant variants were shortlisted based on MAF (<1% in South Asian population); phenotypic correlation and deleteriousness of the mutations were predicted using PolyPhen, SIFT, Mutation Taster, and Splicefinder tools.

Results and Discussion

NGS revealed the presence of 3 clinically relevant variants of unknown significance in 3 genes, namely *INPP5E*, *MBD5*, and *TAF2*. A heterozygous variant was observed in the *MBD5* gene (c.1111C>G; p.Gln371Glu), and homozygous variants were identified in *TAF2* (c.466A>G; p.Lys156Glu) and *INPP5E* (c.1303C>T; p.Arg435Trp). Mutations in *TAF2* are associated with autosomal recessive intellectual disability characterized

Fig. 1. Brain MRI revealing the molar tooth sign, a classical feature of JS/JSRD on axial T1. Deepening of the interpeduncular fossa (*), thick and straight superior cerebellar peduncles (arrows), and hypoplastic vermis are shown.

by postnatal microcephaly, pyramidal signs, thin corpus callosum, and delayed psychomotor development [Hellman-Aharony et al., 2013]. A mutation in the *MBD5* gene is also associated with autosomal dominant intellectual disability [Bonnet et al., 2013], and a mutation in *INPP5E* is known to cause JS/JSRD, which is characterized by the presence of MTS, hypotonia, and psychomotor delay. The variants in *TAF2* and *MBD5* were ruled out as they did not clinically correlate with the proband's presentation; however, the variant in *INPP5E* was considered because it showed correlation with the proband's condition. Brain MRI was advised which showed the presence of MTS of the hind- and midbrain, suggestive of JS. MTS is the result of cerebellar vermis hypoplasia; thick, maloriented superior cerebellar peduncles, and an abnormally deep interpeduncular fossa (Fig. 1). Eye evaluation and abdominal ultrasound were advised to rule out associated findings. Eye evaluation showed no signs of retinopathy, but the ultrasound revealed grade I nephropathy with medullary sponge kidneys, thus confirming the diagnosis JS with renal defects.

The homozygous missense variation c.1303C>T in exon 6 of *INPP5E* results in the substitution of the amino acid arginine with tryptophan at position 435. This change is predicted to be deleterious by in silico analysis with SIFT and PolyPhen tools. The presence of the mutation was confirmed with the help of Sanger sequencing, and targeted mutation analysis (p.Arg435Trp) was carried out for the parents. The chromatograms showed

Table 1. Comparison of clinical findings in our patient and a previously published case

	Proband (n = 1)	Travaglini et al. (2013) (n = 1)
Amino acid change	p.Arg435Trp	p.Arg435Gln
Nucleotide change	c.1303C>T	c.1304G>A
Clinical features		
MTS	+	+
Hypotonia	+	+
PMD	+	+
ID	+	+
Retinopathy	–	+
Coloboma	–	–
Renal	+	–
Hepatic	–	–

ID, intellectual deficiency; MTS, molar tooth sign; PMD, psychomotor delay. +, present; –, absent.

that the variant segregates in the expected pattern in the family with both unaffected parents being heterozygous (Fig. 2a).

The *INPP5E* variant (p.Arg435Trp) has neither been previously reported in dbSNP nor in the 1000 Genome database and has not been reported in the literature. However, a different mutation that changes the amino acid arginine to glutamine at the same position (p.Arg435Gln) has been reported in association with JSRD [Bielas et al., 2009; Travaglini et al., 2013]. This particular change affects the catalytic domain of the protein and results in altered cellular phosphatidylinositol ratios. Bielas et al. [2009] and Travaglini et al. [2013] both reported 3 patients with a homozygous mutation at c.1304G>A in the *INPP5E* gene who were diagnosed to have JSRD. Of these 3 patients, the clinical information was available for only one patient reported by Travaglini et al. [2013]. The information for our proband and the above-mentioned patient has been summarized in (Table 1). However, retinopathy was observed in the patient reported by Travaglini et al. [2013], while it was absent in our patient, probably because of the difference in nucleotide change as this leads to the formation of different proteins.

The crystal structure for the human *INPP5E* protein was available in the Protein Data Bank (PDB code 2XSW). A ball and stick model was prepared using the PyMOL program which revealed that p.Arg435Trp substitution results in significant distortions of hydrogen

Fig. 2. a Sanger sequencing. The red peaks in the proband's chromatogram represent the presence of T nucleotide in a homozygous state, whereas a combination of blue and red peaks is seen in her parents' chromatogram, representing C/T nucleotide in a heterozygous state. **b** Ball and stick model. Analysis of the 2XSW structure using PyMOL revealed that due to the mutation of p.R435W, there was a significant distortion of hydrogen bond interactions.

bond interactions with a potential impact on structure and function (Fig. 2b). These findings provide strong genetic evidence that the identified *INPP5E* variant is likely to be a disease-causing mutation. The *INPP5E* variant identified in this study has been submitted to ClinVar and can be accessed using submission accession (SCV000281757).

Our experience shows that NGS is an effective tool in finding a diagnosis in cases with ill-defined clinical conditions. Here, we describe a novel mutation in the *INPP5E* gene as the likely cause for psychomotor delay, hypotonia, MTS, and nephropathy in a 6-year-old girl.

Statement of Ethics

Informed consent was obtained prior to investigation. The authors have no ethical conflicts to disclose.

Disclosure Statement

The authors declare no conflicts of interest.

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