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ARTICLE

Phenotypic spectrum and prevalence of *INPP5E* mutations in Joubert Syndrome and related disorders

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Joubert syndrome and related disorders (JSRD) are clinically and genetically heterogeneous ciliopathies sharing a peculiar midbrain–hindbrain malformation known as the 'molar tooth sign'. To date, 19 causative genes have been identified, all coding for proteins of the primary cilium. There is clinical and genetic overlap with other ciliopathies, in particular with Meckel syndrome (MKS), that is allelic to JSRD at nine distinct loci. We previously identified the *INPP5E* gene as causative of JSRD in seven families linked to the JBTS1 locus, yet the phenotypic spectrum and prevalence of *INPP5E* mutations in JSRD and MKS remain largely unknown. To address this issue, we performed *INPP5E* mutation analysis in 483 probands, including 408 JSRD patients representative of all clinical subgroups and 75 MKS fetuses. We identified 12 different mutations in 17 probands from 11 JSRD families, with an overall 2.7% mutation frequency among JSRD. The most common clinical presentation among mutated families (7/11, 64%) was Joubert syndrome with ocular involvement (either progressive retinopathy and/or colobomas), while the remaining cases had pure JS. Kidney, liver and skeletal involvement were not observed. None of the MKS fetuses carried *INPP5E* mutations, indicating that the two ciliopathies are not allelic at this locus.
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INTRODUCTION

Joubert syndrome and related disorders (JSRD; MIM213300) are clinically and genetically heterogeneous conditions characterized by cerebellar vermis hypo-dysplasia and a peculiar midbrain–hindbrain malformation, the 'molar tooth sign' (MTS). The typical neurological features of hypotonia, ataxia, psychomotor delay, oculomotor apraxia and neonatal breathing dysregulation are variably associated with a broad spectrum of multiorgan abnormalities, mainly involving the eyes, kidneys and liver.[1] To date, up to 19 genes have been identified with either autosomal recessive (*INPP5E*, *TMEM216*, *AHI1*, *NPHP1*, *CEP290*, *TMEM67*, *RPGRIP1L*, *ARL13B*, *CC2D2A*, *TTC21B*, *KIF7*, *TCTN1*, *TCTN2*, *TMEM237*, *CEP41*, *TMEM138*, *C5orf42*, *TMEM231*) or X-linked inheritance (*OFD1*).[2,3] Intriguingly, all JSRD genes code for proteins of the primary cilium, making these disorders part of the expanding group of ciliopathies.[4] There is large clinical and genetic overlap between JSRD and other ciliopathies such as Meckel syndrome (MKS; MIM249000), isolated nephronophthisis (NPH; MIM256100) and Senior-Loken syndrome (MIM266900). In particular, JSRD and MKS are known to be allelic at nine loci, suggesting that MKS represents the most severe end of the JSRD clinical spectrum.[2] It is estimated that known genes overall account for about half of cases, suggesting further genetic heterogeneity; moreover, genotype–phenotype correlates have been clearly established only for few JSRD-causative genes.[5–9]

In 2009, Jacoby *et al*[10] identified *INPP5E* mutations in a family with MORM syndrome, a rare autosomal recessive condition related

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to Bardet–Biedl syndrome. In the same year, we identified homozygous *INPP5E* mutations in seven consanguineous families genetically linked to the first JSRD locus (JBTS1) on 9q34.[11] To better define the phenotypic spectrum associated with *INPP5E* mutations and to evaluate their potential contribution to MKS, here we performed a comprehensive molecular screening of this gene in nearly 500 probands diagnosed with either JSRD or MKS.

PATIENTS AND METHODS

Patients

Mutation analysis was performed in a total of 483 probands from two cohorts. The first cohort consisted of 408 probands representative of the whole JSRD clinical spectrum, selected from databases located at the IRCCS CSS-Mendel Institute (Rome, Italy), the University of California San Diego (CA, USA) and the Necker Hospital (Paris, France). All patients had neuroradiologically proven MTS. For each patient, a detailed clinical questionnaire filled by the referring clinician allowed to obtain information on the extent of multiorgan involvement. In particular, nearly all patients underwent measurement of renal and hepatic function, abdominal ultrasound, assessment of visual ability and fundoscopy.

The second cohort included 75 fetuses diagnosed with MKS according on established criteria,[12] selected from databases located at the Necker Hospital and the St James's University Hospital (Leeds, UK). Most patients included in this study had undergone mutation analysis of some JSRD/MKS genes as part of published screenings or in subsequent research studies; probands known to carry mutations in other genes were excluded from the screening. In proband COR28, harboring only a single heterozygous *INPP5E* variant, mutations in, the *TMEM216*, *AHI1*, *NPHP1*, *CEP290*, *TMEM67*, *RPGRIP1L* and *TMEM138* genes have been previously excluded. Written informed consent was obtained from all families, and the study was approved by the local ethics committees.

Molecular analysis

Genomic DNA was extracted from peripheral blood lymphocytes or frozen tissue, following standard methods. The whole *INPP5E* (GenBank NM_019892) coding region and splice sites were searched for mutations adopting two distinct strategies. In 308 JSRD probands and in the 75 MKS fetuses, bidirectional sequencing was performed using the BigDye terminator chemistry and an ABI PRISM 3130Xl automated sequencer (Life Technologies, Carlsbad, CA, USA). One-hundred JSRD probands underwent whole-exome sequencing (WES) using an Illumina HiSeq 2000 platform (Illumina, San Diego, CA, USA) and the Agilent SureSelect Human All Exome 50 Mb kit (Agilent, Santa Clara, CA, USA). In patient COR28, in whom only a single heterozygous mutation could be detected, genomic quantitative real-time PCR (qPCR) of all exons was performed to search for deletions or duplications, as described.[13] Primers and PCR conditions are available upon request.

Bioinformatic analysis

For WES, the GATK software was used for variant identification.[14] Thirteen mutation description was checked using the Mutalyzer software (http://www.humgen.nl/mutalyzer/1.0.1); for missense mutations, prediction of pathogenicity was assessed using the PolyPhen-2 software (http://genetics.bwh.harvard.edu/pph2/). Multiple sequence alignments of the human INPP5E protein and its orthologues were generated using ClustalW (http://www.ebi.ac.uk/clustalw/). Public databases were accessed using the following links: dbSNP Build 132 (http://www.ncbi.nlm.nih.gov/projects/SNP/), 1000 Genomes Project (http://www.1000genomes.org/), and Exome Sequencing Project's Exome Variant Server (EVS) (http://evs.gs.washington.edu/EVS/).

RESULTS

The *INPP5E* mutational spectrum

Among JSRD, we identified 12 different *INPP5E* mutations in 17 patients from 11 families, with an overall prevalence of 2.7% (11/408) (Table 1). This figure is likely to be slightly overestimated, as

Table 1 Clinical features of patients with mutations in *INPP5E*

	COR28	*COR64*[a]	*COR176*	*COR199*	*MTI-146*	*MTI-240*	*MTI-430*	*MTI-888*	*MTI-1521*	*JBS-011*	*JBS-024*
No of patients (sex)	1 (F)	3 (M/M/F)	1 (M)	2 (F/F)	1(M)	1(M)	1(F)	2(M/M)	2(M/M)	2 (M/M)	1 (M)
Consang	−	+	?	−	+	+	+	+	+	−	+
Country of origin	Italy	Switzerland	Italy	Italy	Israel	Afghanistan	Turkey	Egypt[b]	Egypt[b]	Algeria	Turkey
Nucleotide change (exon)	c.1420T>C (7) ?	c.1277C>A (5) hom	c.1629C>G (8) hom	c.907G>A (2) c.1753C>T (9)	c.1035G>C (4) hom	c.856G>A (2) hom	c.1304G>A (6) hom	c.1921T>C (10) hom	c.1921T>C (10) hom	c.1600T>G (8) c.1862G>A (10)	c.1688G>A (9) hom
Amino acid change	p.W474R +++ ?	p.T426N++ hom	p.Y543X hom	p.V303M+ p.R585C+++	p.R345S++ hom	p.G286R+++ hom	p.R435Q++ hom	p.C641R+++ hom	p.C641R+++ hom	p.Y534D+++ p.R621Q++	p.R563H++ hom
Age at examination	2 Years	11 Years/ 15 years/ 18 years	14 Years	15 Years/ 20 years	21 Months	3 Years	1.8 Years	1 Year/ 8 years	3 Years/ 1 year	2 Years/ 6 years	4 Years
Neurological:											
MTS	y	y/y/y	y	y/y	y	y	y	y/y	y/y	y/y	y
Hypotonia	y	y/y/y	y	y/y	y	y	y	y/y	y/mild	y/y	y
Abnormal breathing	n	?/n/n	n	n/n	y	y	y	y/y	y/n	n/n	y
PMD	Mild	y/y/n	y	y/y	y	y	y	y/y	y/mild	y/y	Severe
ID	y	y/y/n	y	y/y	y	y	y	y/y	y/mild	?/y	y
OMA	y	y/y/y	y	y/y	y	y	y	y/y	y/y	y/y	?
Other	Arachnoid cyst				Bruxism				Ptosis/n		Aganglionic colon
Ocular											
Retinopathy	n	n/n/n	y	y/y	y	n	y	y/y	y/n	?/n	?
Coloboma	n	n/n/n	y	n/y	n	y	n	y/y	n/n	n/n	?
Renal	n	n/n/n	n	n/n	n	n	n	n/n	n/n	LK ectopia	n
Hepatic	n	n/n/n	n	n/n	n	n	n	n/n	n/n	?/n	n

Abbreviations: F, female; ID, intellectual deficiency; LK, left kidney; M, male; MTS, molar tooth sign; n, no; OMA, oculomotor apraxia; PMD, psychomotor delay; Ret, retardation; y, yes.
[a]A detailed cognitive assessment of patients from this family has been previously reported.[26] Polyphen prediction for missense mutations: + benign; ++ possibly damaging; +++ probably damaging.
[b]Apparently unrelated families originating from different Egypt regions.

we excluded from the screening a subset of about 50 probands known to carry pathogenic mutations in other genes.

Ten mutations were novel, whereas two (p.R435Q, pR563H) had been previously reported.[11] Mutations were homozygous in eight families and compound heterozygous in two. In COR28, only a single mutated allele was found, and genomic qPCR failed to identify deletions or duplications of *INPP5E* exons. Only one mutation was nonsense (p.Y543X), while the remaining 11 were missense. Nearly all changes were predicted as probably or possibly damaging by Polyphen-2, clustered within the enzymatically active phosphatase domain of the protein and affected residues that were highly conserved among different species (Figure 1). None of the newly identified mutations were encountered among 200 control chromosomes, nor have been previously reported as polymorphisms or rare variants in public databases such as dbSNP, 1000 Genomes, and EVS. All mutations segregated with the disease within families, insofar parents were always heterozygous for one *INPP5E* mutation while all affected members carried two mutations (with the exception of COR28). In the cohort of 75 MKS fetuses, no pathogenic mutations were found.

Phenotypes associated with *INPP5E* mutations

Detailed clinical features of patients bearing *INPP5E* mutations are described in Table 1. Seven out of eleven families (64%) presented a phenotype of JS with ocular involvement, consisting of either progressive retinopathy, chorio-retinal colobomas, or both, while the other four families had pure Joubert syndrome, only characterized by neurological signs.

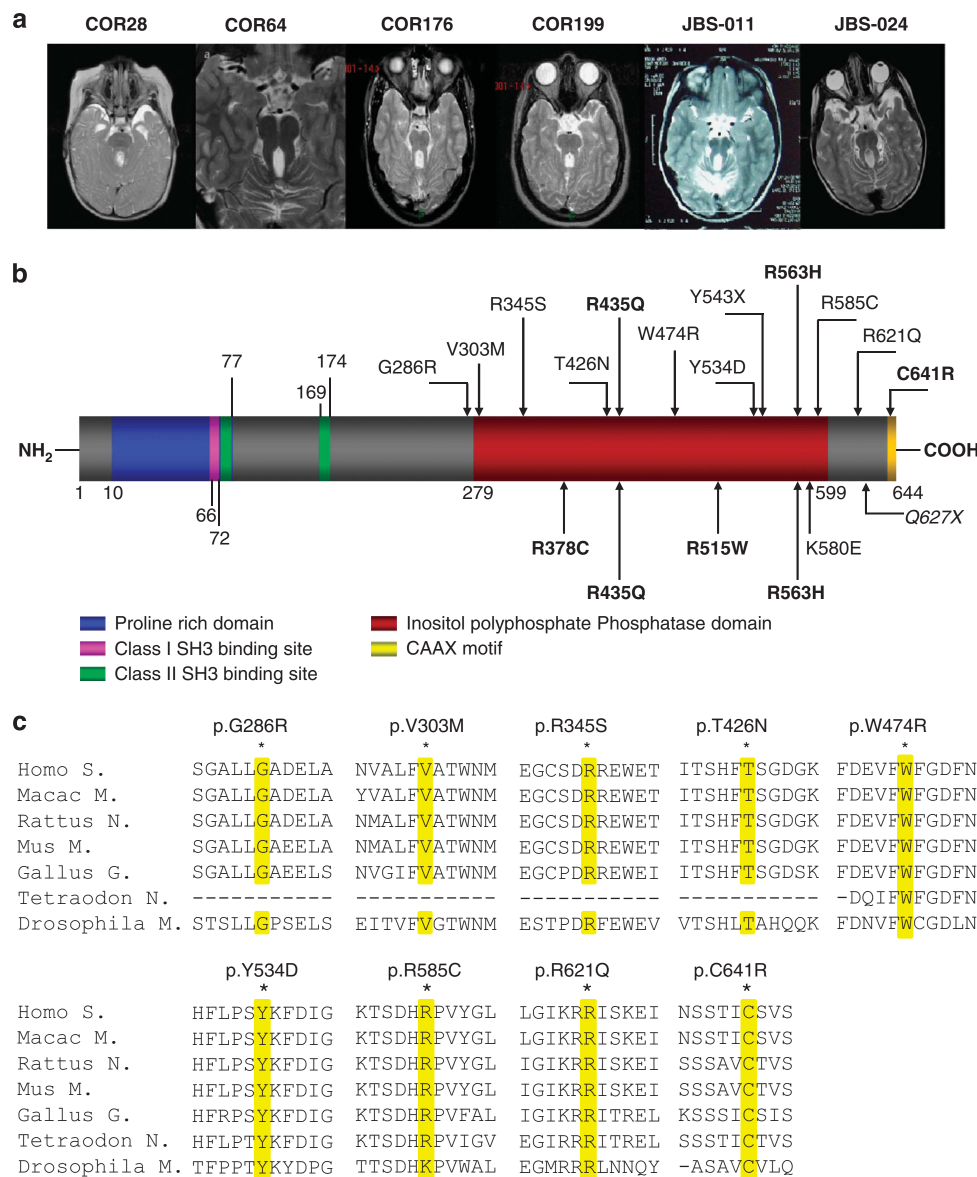

Figure 1 Mutations identified in *INPP5E*. (**a**) Axial magnetic resonance imaging from six probands with mutations in *INPP5E*, showing the MTS. (**b**) Schematic representation of the INPP5E protein consisting of four domains, including a proline rich domain, two small SH3 domains, a large conserved 300-amino-acid catalytic domain and a CAAX domain at C-terminus of the protein. Mutations identified in this article are reported above the panel, while previously described mutations are shown below (the only MORM-associated mutation in shown in *italic*). Recurrent mutations are shown in bold. (**c**) Conservation across species (shaded in yellow) of residues affected by the nine novel missense variants.

Interestingly, intra-familial variability was observed in some families with multiple affected siblings. For instance, the affected sibs in families COR199 and MTI-1521 were discordant for the presence of either chorio-retinal coloboma or retinopathy. Similarly, the severity of psychomotor delay varied widely in families COR64 and MTI-1521, in which one affected sib presented mild or even absent intellectual deficiency, while the other sibling(s) had a full blown neurological phenotype.

DISCUSSION

Here, we present the first large-scale molecular screening of the *INPP5E* gene in nearly 500 patients with diagnosis of JSRD or MKS, two ciliopathies allelic at nine loci. Among JSRD, we identified 12 *INPP5E* mutations of which 10 novel, raising to 16 the number of distinct pathogenic changes so far reported.[10–11] We describe for the first time a homozygous nonsense mutation (p.Y543X), resulting in the production of a truncated protein lacking the final part of the catalytic domain and the C-terminus transmembrane domain. All the remaining mutations are missense changes affecting evolutionarily conserved amino-acid residues clustered within or flanking the enzymatically active phosphatase domain (Figure 1). INPP5E is a member of the 5-ptase family, a class of enzymes that degrade 5-position phosphates from Phosphoinositides (PtdIns), thus regulating diverse cellular processes such as synaptic vesicle recycling, insulin signaling, and embryonic development.[15] In particular, INPP5E has been shown to promote ciliary stabilization, and some mutations have been previously shown to alter the INPP5E enzymatic activity and promote premature cilia destabilization in response to specific stimuli.[10–11]

Our data indicate a mutation prevalence of about 2.7% among JSRD, while no pathogenic changes were detected in 75 MKS fetuses, suggesting that *INPP5E* is not causative of the MKS phenotype. Lack of allelism between these two conditions has been already described for other ciliary genes such as *ARL13B* and *CEP41*, that are mutated only in JSRD.[16,17] It can be postulated that, at least during embryonic development, the functioning of some ciliary proteins could be less crucial than others or compensated by other proteins acting in the same pathway, insofar even their complete loss of function would not result in a lethal phenotype such as MKS. In fact, the only patient harboring an *INPP5E* homozygous truncating mutation presented a relatively mild phenotype of JS plus retinopathy and colobomas, arguing against a specific correlation between the protein residual function and phenotypic severity.

Including the present study, a total of 33 *INPP5E*-mutated patients from 18 JSRD families have been described. Overall, the most common presentation appears to be JS plus ocular involvement, characterized by progressive retinopathy and/or chorio-retinal colobomas (10/18, 56%). Interestingly, in these patients retinal disease was never severe; in fact, none of them presented with Leber congenital amaurosis, that is the typical retinopathy found in patients mutated in *CEP290*.[5] Pure JS is also a frequent presentation, occurring in five families (28%), while other phenotypes such as JS with liver disease (COACH syndrome) and JS with renal disease are extremely rare, being reported only in two and one families, respectively. However, it must be noted that age of examination of some patients was too young to safely exclude the development of a progressive renal disease such as NPH. The *INPP5E* phenotypic spectrum appears to largely overlap with that related to *AHI1* mutations;[18] conversely, none of the *INPP5E*-mutated patients presented polydactyly or encephalocele, two features that are often associated with mutations in genes also causative of MKS, such as *TMEM216*, *CEP290*, *TMEM67* or *RPGRIP1L*.[5,19–21]

In one patient with pure JS (COR28), only a single heterozygous *INPP5E* mutation could be detected, despite complete sequencing of the coding regions and canonical splice sites, and search for genomic rearrangements. Although we cannot exclude the possibility that a second pathogenic mutation resides within intronic or regulatory regions of the gene, it is also plausible that the identified change could act as a genetic modifier of the clinical phenotype in an oligogenic context, and that digenic mutations may reside in another gene. This intriguing mechanism has been already postulated for several ciliopathies including Bardet–Biedl syndrome, NPH and even JSRD,[22–25] and could also help explain the intra-familial variability observed in some *INPP5E*-mutated families. To this end, the systematic genetic screening of multiple ciliopathy genes based on innovative technologies such as next-generation-sequencing is expected to give a main contribution to clarify the molecular basis underlying the clinical complexity of JSRD and other ciliopathies.

NOTE ADDED IN PROOF

While this paper was being reviewed, two novel genes causative of JSRD have been reported. These are *TCTN3* (Thomas *et al*[27]) and *ZNF423* (Chaki *et al*[28]).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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APPENDIX

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