

ARTICLE

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

Lorena Travaglini^{1,2}, Francesco Brancati^{1,3}, Jennifer Silhavy^{4,5}, Miriam Iannicelli¹, Elizabeth Nickerson⁶, Nadia Elkhartoufi⁷, Eric Scott^{4,5}, Emily Spencer^{4,5}, Stacey Gabriel⁶, Sophie Thomas⁷, Bruria Ben-Zeev⁸, Enrico Bertini², Eugen Boltshauser⁹, Malika Chaouch¹⁰, Maria Roberta Cilio¹¹, Mirjam M de Jong¹², Hulya Kayserili¹³, Gonul Ogur¹⁴, Andrea Poretti^{9,15}, Sabrina Signorini¹⁶, Graziella Uziel¹⁷, Maha S Zaki¹⁸, the International JSRD Study Group, Colin Johnson¹⁹, Tania Attié-Bitach⁷, Joseph G Gleeson^{4,5} and Enza Maria Valente^{*,1,20}

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.

European Journal of Human Genetics (2013) 21, 1074–1078; doi:10.1038/ejhg.2012.305; published online 6 February 2013

Keywords: *INPP5E*; Joubert syndrome and related disorders; Meckel syndrome; ciliopathies

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*, *AH11*, *NPH1*, *CEP290*, *TMEM67*, *RPGRIPL1*, *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

¹IRCCS Casa Sollievo della Sofferenza, Mendel Laboratory San Giovanni Rotondo, San Giovanni Rotondo, Italy; ²Unit of Molecular Medicine for Neuromuscular and Neurodegenerative Diseases, Bambino Gesù Children’s Hospital, IRCCS, Rome, Italy; ³Department of Biopathology and Diagnostic Imaging, Tor Vergata University, Rome, Italy; ⁴Department of Neurosciences, Howard Hughes Medical Institute, University of California, San Diego, La Jolla, CA, USA; ⁵Neurogenetics Laboratory, Institute for Genomic Medicine, Department of Pediatrics, University of California, San Diego, La Jolla, CA, USA; ⁶The Broad Institute of MIT and Harvard, Cambridge, MA, USA; ⁷Département de Génétique, Institut National de la Santé et de la Recherche Médicale (INSERM) U781, Hôpital Necker–Enfants Malades, Université Paris Descartes, Paris, France; ⁸Edmond and Lilly Safra Children’s Hospital, Sheba Medical Center, Ramat Gan, Israel; ⁹Department of Pediatric Neurology, University Children’s Hospital of Zürich, Zurich, Switzerland; ¹⁰Service de Neurologie, Centre Hospitalier Universitaire Ben-Aknoun, Algiers, Algérie; ¹¹Division of Neurology, Bambino Gesù Children’s Hospital, IRCCS, Rome, Italy; ¹²Department of Genetics, University Medical Centre Groningen, University of Groningen, Groningen, The Netherlands; ¹³Medical Genetics Department, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey; ¹⁴Ondokuz Mayıs University Medical Faculty, Departments of Medical and Pediatric Genetics, Samsun, Turkey; ¹⁵Division of Pediatric Radiology, Russell H Morgan Department of Radiology and Radiological Science, The Johns Hopkins University School of Medicine, Baltimore, MD, USA; ¹⁶Unit of Child Neurology and Psychiatry, C Mondino National Institute of Neurology, IRCCS, Pavia, Italy; ¹⁷Department of Child Neurology, Fondazione IRCCS Istituto Neurologico C Besta, Milan, Italy; ¹⁸Clinical Genetics Department, Human Genetics and Genome Research Division, National Research Centre, Cairo, Egypt; ¹⁹Section of Ophthalmology and Neurosciences, Wellcome Trust Brenner Building, Leeds Institute of Molecular Medicine, St James’s University Hospital, Leeds, UK; ²⁰Department of Medicine and Surgery, University of Salerno, Salerno, Italy

*Correspondence: Professor EM Valente, Neurogenetics Unit, CSS-Mendel Institute, Viale Regina Margherita 261, Rome 00198, Italy. Tel: +39 06 4416 0537, Fax: +39 06 4416 0548; E-mail: e.valente@css-mendel.it

Received 20 July 2012; revised 3 October 2012; accepted 5 December 2012; published online 6 February 2013

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 to 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*, *RPGRIPL1* 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) p.V303M + p.R585C + +	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) p.Y534D + + p.R621Q + +	c.1688G>A (9) hom
Amino acid change	p.W474R + + +	p.T426N + + hom	p.Y543X hom	p.R585C + + +	p.R345S + hom	p.G286R + + +	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/n/n	y	y/n	y	y	y	y/y	y/mild	y/n	Severe
ID	y	y/n/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	?/y
Other	Arachnoid cyst				Bruxism				Ptoisis/n		Agangiomatic 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
^aA detailed cognitive assessment of patients from this family has been previously reported.²⁵ Polyphen prediction for missense mutations: + benign; ++ possibly damaging; +++ probably damaging.
^bApparently 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, p.R563H) 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.

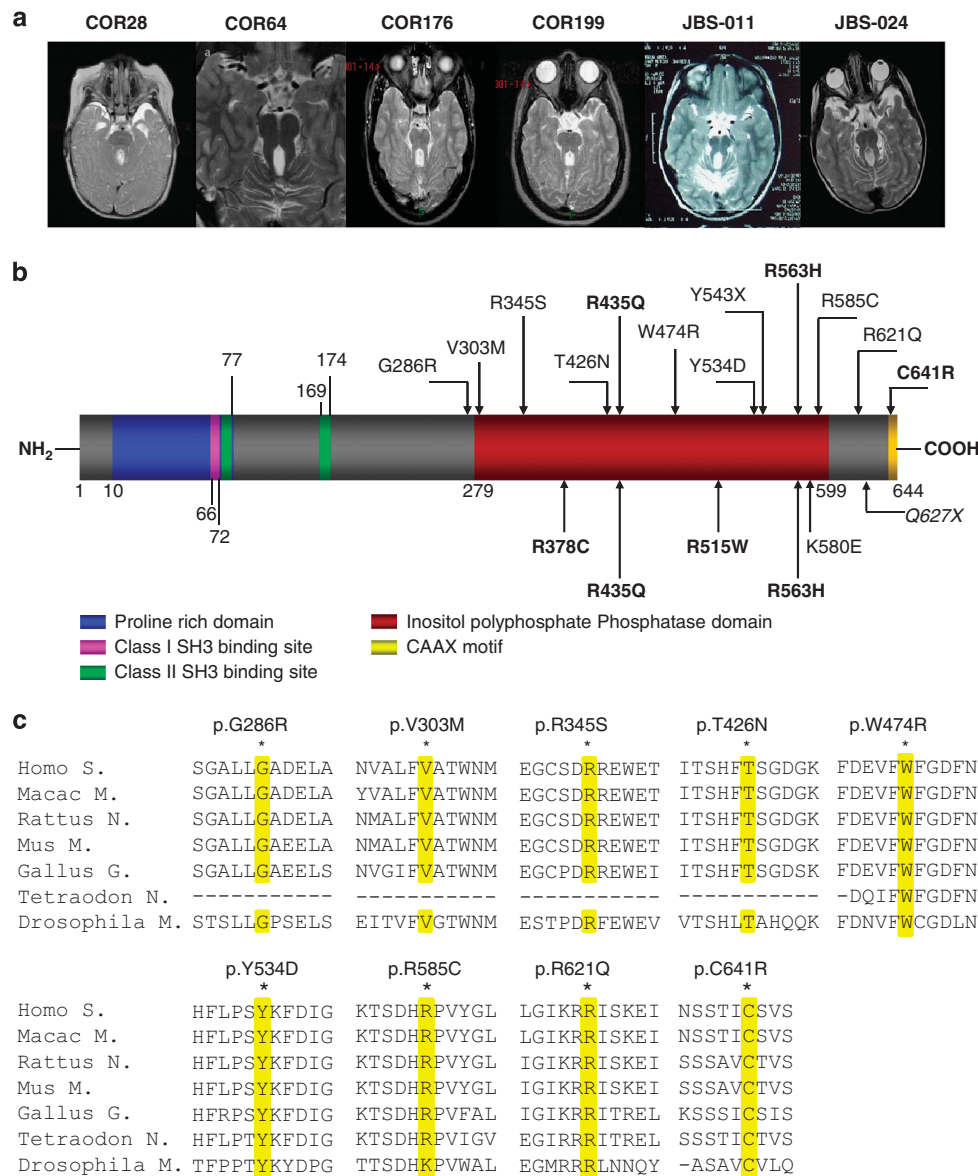


Figure 1 Mutations identified in *INPP5E*. (a) Axial magnetic resonance imaging from six probands with mutations in *INPP5E*, showing the MTS. (b) Schematic representation of the *INPP5E* protein consisting of four domains, including a proline rich domain, two small SH3 domains, a large conserved 300-amino-acid 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 is 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.

ACKNOWLEDGEMENTS

This work was partly supported with grants from the Italian Ministry of Health (Ricerca Corrente 2012 to EMV, Ricerca Finalizzata 2009 to EB and EMV), the Italian Telethon Foundation (GGP08045 to EB and EMV), the European Research Council (Starting Grant 260888 to EMV), the National Institute of Health (R01NS048453 to JGG), the Broad Institute (U54HG003067 to Eric Lander) for sequencing support and analysis. We thank Dr Celine Gomes and Professor Arnold Munnich for referring patients.

- 1 Brancati F, Dallapiccola B, Valente EM: Joubert Syndrome and related disorders. *Orphanet J Rare Dis* 2010; **5**: 20.
- 2 Parisi M, Glass I: Joubert Syndrome and Related Disorders: *GeneReviews*. <http://www.ncbi.nlm.nih.gov/books/NBK1325/> Initial Posting: 9 July 2003; Last Revision: 14 June 2012.
- 3 Srour M, Hamdan FF, Schwartzentruber JA *et al*: Mutation in *TMEM231* cause Joubert Syndrome in French Canadians. *J Med Genet* 2012; **49**: 636–641.
- 4 Novarino G, Akizu N, Gleeson JG: Modeling human disease in humans: the ciliopathies. *Cell* 2011; **147**: 70–79.
- 5 Brancati F, Barrano G, Silhavy JL *et al*: CEP290 mutations are frequently identified in the oculo-renal form of Joubert syndrome-related disorders. *Am J Hum Genet* 2007; **81**: 104–113.
- 6 Brancati F, Iannicelli M, Travaglini L *et al*: MKS3/TMEM67 mutations are a major cause of COACH Syndrome, a Joubert Syndrome related disorder with liver involvement. *Hum Mutat* 2009; **30**: E432–E442.
- 7 Castori M, Valente EM, Donati MA *et al*: NPHP1 gene deletion is a rare cause of Joubert syndrome related disorders. *J Med Genet* 2005; **42**: e9.
- 8 Bachmann-Gagescu R, Ishak GE, Dempsey JC *et al*: Genotype-phenotype correlation in CC2D2A-related Joubert syndrome reveals an association with ventriculomegaly and seizures. *J Med Genet* 2012; **49**: 126–137.
- 9 Doherty D, Parisi MA, Finn LS *et al*: Mutations in 3 genes (MKS3, CC2D2A and RPGRIP1L) cause COACH syndrome (Joubert syndrome with congenital hepatic fibrosis). *J Med Genet* 2010; **47**: 8–21.
- 10 Jacoby M, Cox JJ, Gayral S *et al*: INPP5E mutations cause primary cilium signaling defects, ciliary instability and ciliopathies in human and mouse. *Nat Genet* 2009; **41**: 1027–1031.
- 11 Bielas SL, Silhavy JL, Brancati F *et al*: Mutations in INPP5E, encoding inositol polyphosphate-5-phosphatase E, link phosphatidylinositol signaling to the ciliopathies. *Nat Genet* 2009; **41**: 1032–1036.
- 12 Salonen R: The Meckel syndrome: clinicopathological findings in 67 patients. *Am J Med Genet* 1984; **18**: 671–689.

- 13 Travaglini L, Brancati F, Attie-Bitach T *et al*: Expanding CEP290 mutational spectrum in ciliopathies. *Am J Med Genet* 2009; **149A**: 2173–2180.
- 14 DePristo MA, Banks E, Poplin R *et al*: A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat Genet* 2011; **43**: 491–498.
- 15 Dyson JM, Fedele CG, Davies EM, Becanovic J, Mitchell CA: Phosphoinositide phosphatases: just as important as the kinases. *Subcell Biochem* 2012; **58**: 215–279.
- 16 Cantagrel V, Silhavy JL, Bielas SL *et al*: Mutations in the cilia gene ARL13B lead to the classical form of Joubert syndrome. *Am J Hum Genet* 2008; **83**: 170–179.
- 17 Lee JE, Silhavy JL, Zaki MS *et al*: CEP41 is mutated in Joubert syndrome and is required for tubulin glutamylation at the cilium. *Nat Genet* 2012; **44**: 193–199.
- 18 Valente EM, Brancati F, Silhavy JL *et al*: AHI1 gene mutations cause specific forms of Joubert syndrome-related disorders. *Ann Neurol* 2006; **59**: 527–534.
- 19 Valente EM, Logan CV, Mougou-Zerelli S *et al*: Mutations in TMEM216 perturb ciliogenesis and cause Joubert, Meckel and related syndromes. *Nat Genet* 2010; **42**: 619–625.
- 20 Iannicelli M, Brancati F, Mougou-Zerelli S *et al*: Novel TMEM67 mutations and genotype-phenotype correlates in meckelin-related ciliopathies. *Hum Mutat* 2010; **31**: E1319–E1331.
- 21 Arts HH, Doherty D, van Beersum SE *et al*: Mutations in the gene encoding the basal body protein RPGRIP1L, a nephrocystin-4 interactor, cause Joubert syndrome. *Nat Genet* 2007; **39**: 882–888.
- 22 Khanna H, Davis EE, Murga-Zamalloa CA *et al*: A common allele in RPGRIP1L is a modifier of retinal degeneration in ciliopathies. *Nat Genet* 2009; **41**: 739–745.
- 23 Louie CM, Caridi G, Lopes VS *et al*: AHI1 is required for photoreceptor outer segment development and is a modifier for retinal degeneration in nephronophthisis. *Nat Genet* 2010; **42**: 175–180.
- 24 Davis EE, Zhang Q, Liu Q *et al*: TTC21B contributes both causal and modifying alleles across the ciliopathy spectrum. *Nat Genet* 2011; **43**: 189–196.
- 25 Katsanis N, Ansley SJ, Badano JL *et al*: Triallelic inheritance in Bardet-Biedl syndrome, a Mendelian recessive disorder. *Science* 2001; **293**: 2256–2259.
- 26 Poretti A, Dietrich Alber F, Brancati F, Dallapiccola B, Valente EM, Boltshauser E: Normal cognitive functions in joubert syndrome. *Neuropediatrics* 2009; **40**: 287–290.
- 27 Thomas S *et al*: TCTN3 mutations cause Mohr-Majewski syndrome. *Am J Hum Genet* 2012; **91**: 372–378.
- 28 Chaki M *et al*: Exome capture reveals ZNF423 and CEP164 mutations, linking renal ciliopathies to DNA damage response signaling. *Cell* 2012; **150**: 533–548.

APPENDIX

Other members of the International JSRD Study Group are: L Ali Pacha (Alger, Algeria); A Zankl (Brisbane, Australia); R Leventer (Parkville, Australia); P Grattan-Smith (Sydney, Australia); A Janecke (Innsbruck, Austria); J Koch (Salzburg, Austria); M Freilinger (Vienna, Austria); M D'Hooghe (Brugge, Belgium); Y Sznajer, C Vilain (Bruxelles, Belgium); R Van Coster (Ghent, Belgium); L Demerleir (Brussels, Belgium); K Dias, C Moco, A Moreira (Porto Alegre, Brazil); C Ae Kim (Sao Paulo, Brazil); G Maegawa (Toronto, Canada); I Dakovic, D Loncarevic, V Mejaski-Bosnjak, D Petkovic (Zagreb, Croatia); GMH Abdel-Salam, A Abdel-Aleem, (Cairo, Egypt); I Marti, JM Pinard, S Quijano-Roy (Garches, France); S Sigaudy (Marseille, France); P de Lonlay, S Romano, A Verloes (Paris, France); R Touraine (St Etienne, France); M Koenig, H Dollfus, E Flori, M Fradin, C Lagier-Tourenne, J Messer (Strasbourg, France); P Collignon (Toulon, France); JM Penzies (Augsburg, Germany); C Bussmann (Heidelberg, Germany); A Merckenschlager (Leipzig, Germany); H Philippi (Mainz, Germany); G Kurlemann (Munster, Germany); K Grundmann (Tubingen, Germany); C Dacou-Voutetakis, S Kitsiou Tzeli, R Pons (Athens, Greece); J Jerney (Budapest, Hungary); S Halldorsson, J Johannsdottir, P Ludvigsson (Reykjavik, Iceland); SR Phadke (Lucknow, India); KM Girisha (Manipal, India); H Doshi, V Udani (Mumbai, India); M Kaul (Rajkot, India); B Stuart (Dublin, Ireland); A Magee (Belfast, Northern Ireland); R Spiegel, S Shalev (Afula, Israel); H Mandel (Haifa, Israel); D Lev, M Michelson (Holon, Israel); M Idit (Petach-Tikva, Israel); B Ben-Zeev (Ramat-Gan, Israel); R Gershoni-Baruch (Rambam, Israel); A Ficcadenti (Ancona, Italy); R Fischetto, M Gentile (Bari, Italy); M Della Monica (Benevento, Italy); M Pezzani (Bergamo, Italy); C Graziano, M Seri (Bologna); F Benedicenti, F Stanzial (Bolzano, Italy); R Borgatti, R Romaniello (Bosisio Parini, Italy); P Accorsi, S Battaglia, E Fazzi, L Giordano, L Pinelli (Brescia, Italy); L Boccone (Cagliari, Italy); R Barone, G Sorge (Catania, Italy); E Briatore (Cuneo, Italy); S Bigoni, A Ferlini (Ferrara, Italy); MA Donati (Florence, Italy); R Biancheri, G Caridi, MT Divizia, F Faravelli, G Ghiggeri, M Mirabelli, A Pessagno, A Rossi, V Uliana (Genoa, Italy); M Amorini, M Briguglio, S Briuglia, CD Salpietro, G Tortorella (Messina, Italy); A Adami, MT Bonati, P Castorina, S D'Arrigo, F Lalatta, G Marra, I Moroni, C Pantaleoni, D Riva, B Scelsa, L Spaccini (Milan, Italy); E Del Giudice (Napoli, Italy); K Ludwig, A Permian, A Suppiej (Padova, Italy); C Macaluso (Parma, Italy);

A Pichiecchio (Pavia, Italy); R Battini (Pisa, Italy); M Di Giacomo (Potenza, Italy); M Priolo, P Timpani (Reggio Calabria, Italy); G Pagani (Rho, Italy); ML Di Sabato, F Emma, V Leuzzi, F Mancini, S Majore, A Micalizzi, P Parisi, M Romani, G Stringini, G Zanni (Rome, Italy); L Ulgheri (Sassari, Italy); M Pollazzon, A Renieri (Siena, Italy); E Belligni, E Grosso, I Pieri, M Silengo (Torino, Italy); R Devescovi (Trieste, Italy); D Greco, C Romano (Troina, Italy); M Cazzagon (Udine, Italy); A Simonati (Verona, Italy); AA Al-Tawari, L Bastaki, (Kuwait City, Kuwait); A Mégarbané (Beirut, Lebanon); V Sabolic Avramovska (Skopje, Macedonia); E Said (Msida, Malta); P Stromme (Oslo, Norway); R Koul, A Rajab (Muscat, Oman); M Azam (Islamabad, Pakistan); C Barbot (Oporto, Portugal); MA Salih, B Tabarki (Riyadh, Saudi Arabia); B Jovic-Jakubi (Nis, Serbia); L Martorell Sampol (Barcelona, Spain); B Rodriguez (La Coruna, Spain); I Pascual-Castroviejo (Madrid, Spain); B Gener (Vizcaya, Spain); A Puschmann (Lund, Sweden); L Starck (Stockholm, Sweden); A Capone (Aarau, Switzerland); J Lemke (Bern, Switzerland); J Fluss (Geneva, Switzerland); D Niedrist (Zurich, Switzerland); RCM Hennekam, N Wolf (Amsterdam, The Netherlands); N Gouider-Khouja, I Kraoua (Tunis, Tunisia); S Ceylaner, S Teber (Ankara, Turkey); M Akgul (Izmir, Turkey); B Anlar, S Comu, H Kayserili, A Yüksel (Istanbul, Turkey); M Akcakus, AO Caglayan (Kayseri, Turkey); O Aldemir (Mersin, Turkey); L Al Gazali, L Sztriha (Al Ain, UAE); D Nicholl (Birmingham, UK); CG Woods (Cambridge, UK); C Bennett, J Hurst, E Sheridan (Leeds, UK); A Barnicoat, C Hemingway, M Lees, E Wakeling (London, UK); E Blair (Oxford, UK); S Bernes (Mesa, Arizona, USA); H Sanchez (Fremont, California, USA); AE Clark (Laguna Niguel, California, USA); E DeMarco, C Donahue, E Sherr (San Francisco, California, USA); J Hahn, TD Sanger (Stanford California, USA); TE Gallager (Manoa, Hawaii, USA); C Daugherty (Bangor, Maine, USA); KS Krishnamoorthy, D Sarco, CA Walsh (Boston, Massachusetts, USA); T McKanna (Grand Rapids, Michigan, USA); J Milisa (Albuquerque, New Mexico, USA); WK Chung, DC De Vivo, H Raynes, R Schubert (New York, New York, USA); A Seward (Columbus, Ohio, USA); DG Brooks (Philadelphia, Pennsylvania, USA); A Goldstein (Pittsburg, Pennsylvania, USA); J Caldwell, E Finsecke (Tulsa, Oklahoma, USA); BL Maria (Charleston, South Carolina, USA), K Holden (Mt. Pleasant, South Carolina, USA); RP Cruse, E Karaca (Houston, Texas, USA); KJ Swoboda, D Viskochil (Salt Lake City, Utah, USA), WB Dobyns (Seattle, Washington, USA).