REPORT

The Meckel-Gruber Syndrome Gene, *MKS3,* Is Mutated in Joubert Syndrome

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Joubert syndrome (JS) is an autosomal recessive disorder characterized by cerebellar vermis hypoplasia associated with hypotonia, developmental delay, abnormal respiratory patterns, and abnormal eye movements. The association of retinal dystrophy and renal anomalies defines JS type B. JS is a genetically heterogeneous condition with mutations in two genes, *AHI1* and *CEP290,* identified to date. In addition, *NPHP1* deletions identical to those that cause juvenile nephronophthisis have been identified in a subset of patients with a mild form of cerebellar and brainstem anomaly. Occipital encephalocele and/or polydactyly have occasionally been reported in some patients with JS, and these phenotypic features can also be observed in Meckel-Gruber syndrome (MKS). MKS is a rare, autosomal recessive lethal condition characterized by central nervous system malformations (typically, occipital meningoencephalocele), postaxial polydactyly, multicystic kidney dysplasia, and ductal proliferation in the portal area of the liver. Since there is obvious phenotypic overlap between JS and MKS, we hypothesized that mutations in the recently identified *MKS* genes, *MKS1* on chromosome 17q and *MKS3* on 8q, may be a cause of JS. After mutation analysis of *MKS1* and *MKS3* in a series of patients with JS (*n* = 22), we identified *MKS3* mutations in four patients with JS, thus defining *MKS3* as the sixth JS locus (*JBTS6*). No *MKS1* mutations were identified in this series, suggesting that the allelism is restricted to *MKS3*.

Joubert syndrome (JS [MIM 213300]) is an autosomal recessive, multisystem disease characterized by developmental delay, hypotonia, irregular breathing pattern, eye movement abnormalities,[1] and cerebellar vermis hypoplasia/dysplasia with accompanying brainstem abnormalities visualized on axial images, where the depth of the interpeduncular fossa is greater than that usually seen, with an associated narrowing of the midbrain tegmentum and a thickening of the superior cerebellar peduncles resulting in the "molar tooth sign" (MTS).[2] Other variable features include retinal dystrophy and renal anomalies that define type B of JS, also called "cerebello-oculo-renal" syndrome (CORS). JS is a genetically heterogeneous condition. To date, mutations in two genes have been identified as responsible for JS: *AHI1* (*JBTS3* on chromosome 6q23.3)[3] and, most recently, *CEP290* (*JBTS5* on 12q21.3).[4,5] In addition, an *NPHP1* deletion identical to the one that causes juvenile nephronophthisis (NPHP) has been identified in a subset of patients with NPHP and a mild form of MTS, thus defining *NPHP1* as the fourth JS locus (*JBTS4* on 2q13).[6] Two more loci, *JBTS1/CORS1*[7] and *JBTS2/ CORS2,*[8,9] are mapped to chromosomes 9q34.3 and 11p12-11q13.3, respectively.

Occipital encephalocele and polydactyly have occasionally been reported in some patients with JS. These features are also found in Meckel-Gruber syndrome (MKS [MIM 249000]), a rare autosomal recessive lethal condition characterized by postaxial polydactyly, multicystic kidney dysplasia, liver bile–duct proliferation, hepatic developmental defects, and characteristic CNS malformations. In typical cases of MKS, these include posterior occipital encephalocele, prosencephalic dysgenesis, and rhombic roof dysgenesis, but Dandy-Walker (DW) malformation, hydrocephalus, and agenesis of the corpus callosum are also described as occasional features. MKS is genetically heterogeneous, and three loci have been mapped to 17q23 (*MKS1*), 11q13 (*MKS2*), and 8q24 (*MKS3*). Very recently, two genes have been identified: *MKS1* on 17q in Finnish kindreds[10] and *MKS3* on 8q in families from Pakistan and Oman.[11] The phenotypic overlap between JS and MKS is further supported by the CNS malformations seen in the *Wpk* rat model of MKS, which include agenesis of the corpus callosum and hydrocephalus but not exencephaly.[12] The missense mutation, P394L, seen in the rat *Mks3* gene is presumably a hypomorphic allele because of the mild phenotype and viability of the *Wpk* rat.[11]
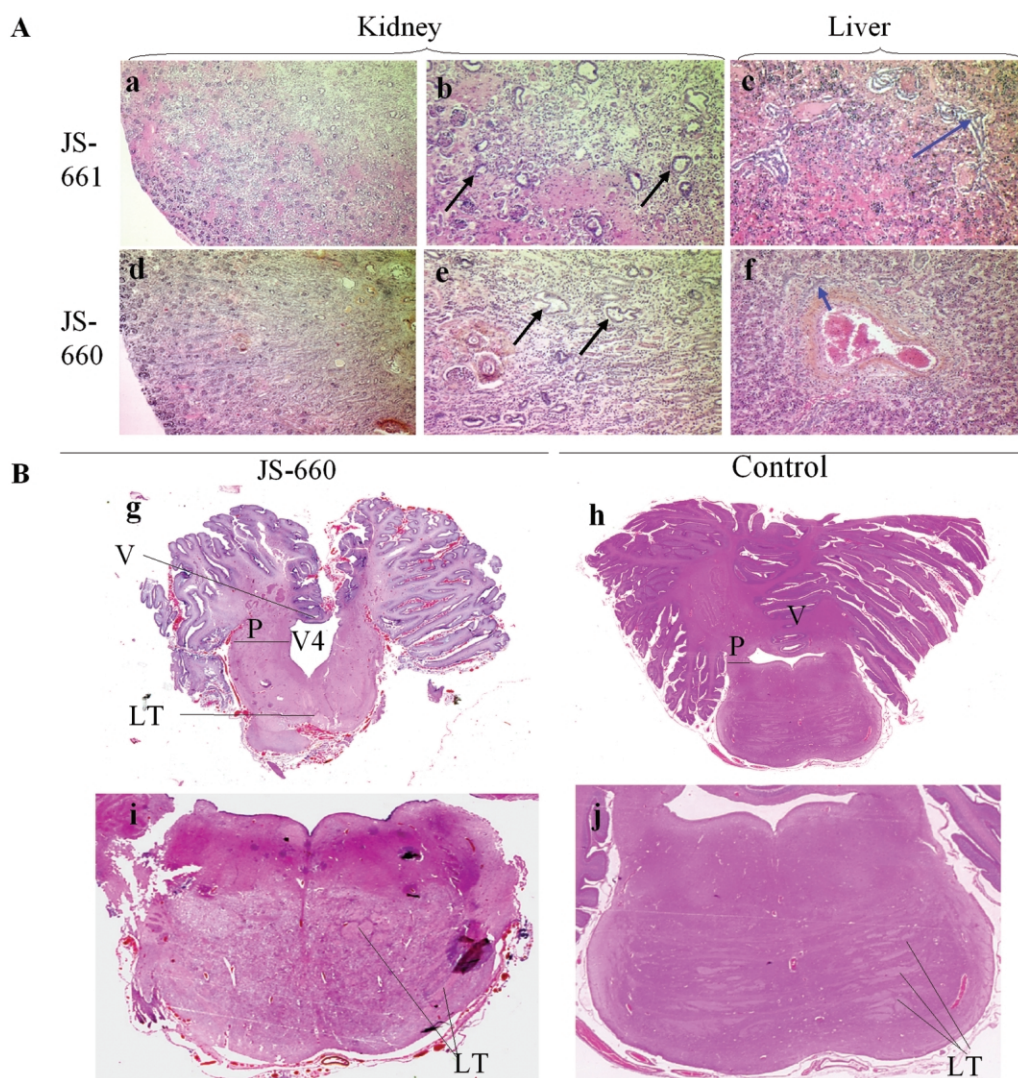
Figure 1. Pathological features of fetal patients with *MKS3* mutations. *A,* Histological pattern of kidney and liver (hematoxylin-eosin-safran staining) in fetuses JS-661 (at 28 WG) and JS-660 (at 30 WG). Kidney is shown at low (*panels a and d*) and high (*panels b and e*) magnification. Liver is shown at high magnification (*panels c and f*). Kidney histology shows conserved corticomedullary organization with 6 to 8 generations of mature glomerules. Microcysts are found in the deep cortex in JS-661. Tubular microcysts in medulla (*black arrows*) are observed in both. Liver histology shows a portal fibrosis with important and diffuse bile-duct proliferation (*blue arrows*) in both fetuses. *B,* Neuropathological findings of brainstem and cerebellum. Transversal section of the brainstem and cerebellum at the level of cerebellar peduncles in fetus JS-660 (*panel g*) compared with a control individual (*panel h*). In panel g, note the inverted molar tooth aspect due to a dysmorphic fourth ventricle (V4) flanked laterally by thick cerebellar peduncles (P). The roof of the fourth ventricle is formed by the remnant of vermian structure (V). Cerebellar folia contain heterotopic Purkinje cells (data not shown). The hypoplastic pons longitudinal tracts (LT) have a chaotic organization, and the transverse fibers are reduced (*panel i*), as compared with a control individual (*panel j*); this is better seen on a caudal level with higher magnification.

While sequencing *MKS1* and *MKS3* genes in 31 unrelated fetuses presenting a cerebro-reno-digital syndrome, which was diagnosed as "Meckel-like" because of the absence of at least one of the MKS diagnostic criteria,[13] we identified *MKS3* mutations in a family with two siblings (fetuses JS-661 and JS-660). In both cases, pregnancies were terminated at 30 wk gestation (WG) and 28 WG, respectively, because of abnormal posterior fossa and hyperechogeneic, enlarged kidneys detected by ultrasound.

Kidney histology showed a conserved corticomedullary organization, with microcysts mainly in the medulla (fig. 1*A* [panels a, b, d, and e]), and liver bile–duct proliferation in both fetuses (fig. 1*A* [panels c and f]). The full fetal autopsies revealed no other malformations. Detailed neuropathological analysis in fetus JS-661 found a cerebellum weighing 14 g (50th percentile for age: 8 g), with a transverse diameter of 33 mm (50th percentile for age: 32 mm). Sagital section of the cerebellum and the brainstem

showed an enlarged, widely open fourth ventricle, hypoplastic/elevated vermis, and thick cerebellar peduncles. Histological study of the cerebellum confirmed vermian hypoplasia reduced to a few folia, fragmented dentate nuclei, and Purkinje cell heterotopias. The brainstem was reduced in size and contained aberrant longitudinal tracts. The inferior olive nuclei were hypoplastic, and the pyramids were asymmetric and reduced in size (data not shown). In fetus JS-660, the cerebellum weighed 10 g (50th percentile for age: 10 g) and had a transverse diameter of 33 mm (50th percentile for age: 35 mm). The transversal section showed a dilated, dysmorphic fourth ventricle flanked laterally by enlarged cerebellar peduncles (mimicking a molar tooth shape) (fig. 1B [panel g]). On histological examination, a remnant of vermian structure was found with fragmented dentate nuclei and Purkinje cell heterotopias. The brainstem was hypoplastic, the longitudinal tracts were aberrant, and the transversal tracts were reduced (fig. 1B [panel i]). We identified two *MKS3* mutations in this family. A missense mutation in exon 15, Y513C, was inherited from the father, and a complex indel mutation (13-bp deletion encompassing the exon22/intron 22 boundary replaced by 2 bp) was inherited from the mother. This 2315_2323+4del13insGG mutation removes the donor splice site and was absent in 228 control chromosomes. The Y513C missense mutation is in the extracellular domain of the protein and affects an amino acid conserved throughout evolution.[11] This mutation was not identified in 214 chromosomes. In silico analysis of the effect of this amino acid substitution on the protein structure and function, with use of the PolyPhen program, predicts that this change is pathogenic, with a high position-specific independent counts (PSIC) score difference >2.[14]

Despite the absence of neurological symptoms required for postnatal diagnosis in these fetuses, JS was highly suspected in this family. We, therefore, questioned whether *MKS* gene mutations could be found in patients with typical JS and sequenced 22 patients with JS with no *NPHP1* deletion. Sequence analysis of *MKS3* revealed mutations in three patients, confirming that *MKS3* is indeed a gene for JS. Mutations and clinical data are summarized in table 1.

JS-05 is a 14-year-old Algerian girl who had consanguineous parents and had already been reported by Romano et al. (designated as "case 2" in their article).[15] She was severely handicapped and presented with hypotonia, severe mental retardation, stereotypic movements, and no independent walking. She had breathing abnormalities but no oculomotor apraxia or abnormal eye movements. Electroretinography and optic disc imaging results were normal. She had no renal or hepatic involvement. Brain imaging showed a cerebellar dysplasia and MTS with no supratentorial anomalies (fig. 2). A homozygous *MKS3* mutation was identified near the donor splice site of intron 23 (IVS23+5G→C). This mutation was absent in 402 chromosomes. Both parents and a healthy sister were heterozygous for the mutation, whereas a healthy brother inherited both normal alleles. RNA was extracted from lymphocytes from the patient and from his father, and RT-PCR with primers located in exons 20 (forward: GTAAGCATATGGAGAACATATTT) and 26 (reverse: CATTGGTTCCATGAATTCCATT) showed a transcript smaller than the expected normal size (754 bp) (fig. 3A). Sequencing confirmed an exon skipping from exons 22 to 24 (fig. 3B). This inframe deletion predicts a protein lacking amino acids 775 to 813, which compose most of the putative coiled-coil domain (amino acids 756 to 912) of the protein.

JS-09 is a mildly affected 7-year-old girl who was also reported elsewhere (as "case 11").[15] She presented with hypotonia, ataxia, oculomotor apraxia, and abnormal eye movements. She had no breathing anomalies or retinal, renal, or hepatic involvement. She had mild motor delay (walked at age 2.5 years) and moderate mental retardation. Brain magnetic resonance imaging (MRI) showed a cerebellar dysplasia with an MTS, an enlarged fourth ventricle, and no supratentorial anomaly (fig. 2). We identified three *MKS3* variations: a donor–splice-site mutation in intron 6 (IVS6+2T→G) inherited from her mother, a missense mutation located in exon 16 (G545E), and a mutation located at the last base of exon 21 (2341G→A). The last two changes were inherited from the father, and both could be deleterious. The G545E mutation concerns a conserved amino acid in vertebrates and is predicted to be pathogenic by the PolyPhen program. The 2341G→A mutation was predicted by the SSF program to decrease the donor–splice-site score. RNA extracted from lymphocyte cell lines of the patients and RT-PCR analysis (performed with the same primers as those for the patient JS-05) did not show an abnormal fragment on electrophoresis. However, the sequencing identified a 16-bp insertion in the transcript, corresponding to the first 16 bp of intron 21 (fig. 3C). An alternative intronic splice site is used when the mutated allele is present. This frameshift mutation predicts a truncated protein with a premature stop codon 14 aa downstream. Whereas the maternal IVS6+2T→G and the paternal G545E mutations were not identified in 380 and 392 chromosomes, respectively, the 2341G→A variation was identified once (1/332 control chromosomes). Since 2341G→A was found in the heterozygote state in the patient, we do not know if this mutation retains some normal splicing. It is, therefore, unclear whether the pathogenic paternal allele results from the G545E missense mutation, the 2341G→A splice mutation, or a combination of the two variations.

Patient NPH-786 is a 7-year-old boy presenting developmental delay, cerebellar ataxia, abnormal breathing, and vermis agenesis. No MRI was performed, and the presence of an MTS or vermis dysgenesis could not be assessed. Since he did not show cystic dilatation of the posterior fossa, a DW malformation was ruled out. The association of hyperechogenic kidneys with cysts and severe hepatic involvement with the vermis agenesis led to the diagnosis

Table 1. Clinical Data of Patients with JS with *MKS3* Mutations

Patient and Nucleotide Change	Exon	Predicted Effect on Protein	Family	Age	Origin	Kidney	Liver	Ocular	CNS
						Phenotypic Features[a]			
JS-661:			1	28 WG	France	Microcysts	HF, BDP	−	CVH, open V4, heterotopias, brainstem anomalies
1538A→G	15	Y513C							
2315_2323+4del13insGG	22	Splice							
JS-660:			1	30 WG	France	Microcysts	HF, BDP	−	CVH, open V4, heterotopias, brainstem anomalies
1538A→G	15	Y513C							
2315_2323+4del13insGG	22	Splice							
JS-05:			2	14 years	Algeria	−	−	−	HT, MR, BA, CVH, MTS
IVS23+5G→C homozygous	23	I775_A813del							
JS-09:			3	7 years	France	−	−	OMA	HT, AT, CVH, enlarged V4, MTS
IVS6+2T→G	6	Splice							
1634G→A	16	G545E							
2341G→A	21	Q747fsX761							
NPH-786:			4	7 years	France	Microcysts	Liver disease	−	AT, MR, BA, CVH, no MRI
637C→T	6	R213C							
2132A→C	21	D711A							

[a] AT = ataxia; BDP = bile-duct proliferation of liver; BA = breathing anomalies; CVH = cerebellar hypoplasia; HF = hepatic fibrosis; HT = hypotonia; MR = mental retardation; OMA = oculomotor apraxia; V4 = fourth ventricle.
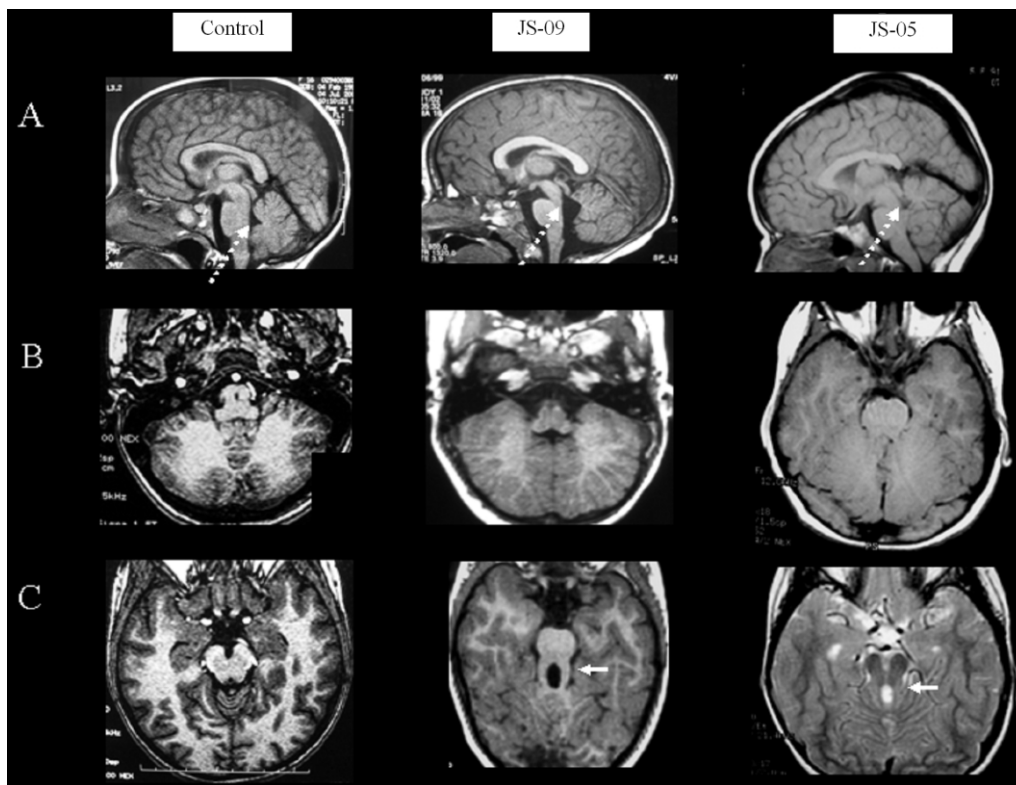
Figure 2. Brain MRI of a normal control individual, an affected girl aged 7 years (JS-09), and an affected girl aged 14 years (JS-05) carrying *MKS3* mutations. *A,* Sagittal MRI in JS-09 shows a dilated fourth ventricle slightly superiorly displaced (*white arrows*). The fourth ventricle in JS-05 was normally sized but superiorly displaced. Both patients JS-09 and JS-05 show a superior vermian dysplasia. The middle and inferior segments of the vermis are hypoplastic. *B,* Axial images at the level of the cerebellar hemispheres show that the middle and inferior segments of the vermis are hypoplastic in patients JS-09 and JS-05; the two hemispheres are not separated by any vermian structure. Patient JS-09 had also an hemispheric cerebellar dysplasia. *C,* Axial images showing the MTS (*white arrow*) at the level of the superior cerebellar peduncles in patients JS-09 and JS-05.

of JS in this patient. We identified two missense mutations, in exons 6 (R213C) and 21 (D711A). Both substitutions are conserved in mammals[11] and were absent in 380 and 332 chromosomes, respectively. Parental DNA samples were not available for mutation analysis. They are both predicted to be pathogenic by the PolyPhen program, with a PSIC score difference of 1.8 for both changes. In addition, D711 is the first amino acid after the third putative transmembrane domain of meckelin,[11] and the substitution of a hydrophobic amino acid, A711, may cause the aberrant inclusion of this alanine in the transmembrane helix, as predicted by the TMHMM Server.

The identification of *MKS3* mutations in three patients with JS defines *MKS3* as the sixth JS locus (*JBTS6*). Despite a relatively small JS series, mutations in the *MKS3* gene could account for ~10% (3/22) of patients with JS. No *MKS1* mutation was identified, suggesting that allelism between JS and MKS is specific to the *MKS3* locus.

MKS and JS are disorders featuring a hindbrain malformation and renal and hepatic involvement and are generally included in the "congenital hepatorenal fibrocystic" group of diseases.[16] Although a severe and lethal cystic

kidney dysplasia is a consistent feature of MKS, nephronophthisis or cystic dysplastic kidneys are associated with JS in some patients, defining the CORS group of JS-related disorders. Concerning the brain malformation, cerebellar vermis agenesis is the cardinal feature of JS, and occipital encephalocele is the most-frequent MKS brain anomaly. Interestingly, some patients with a cerebellar vermis agenesis also present an occipital encephalocele (reviewed by Satran et al.[17]), and intrafamilial variability has been reported in discordant monozygous twins presenting JS with or without encephalocele.[18] DW malformation has been described in both disorders. In MKS, sibs were reported with either occipital encephalocele or DW malformation[19] or both.[20] Patients with JS and DW malformations or variants have also been discussed in many cases.[21–23] However, these patients did not undergo brain MRI, and the state of the brainstem is unknown. Also, the diagnosis of DW malformation was based on either ultrasound or neuropathological examination results. The neuropathological definition of DW malformation differs from the radiological definition and includes partial or complete vermis agenesis with cystic dilatation of posterior fossa, with or
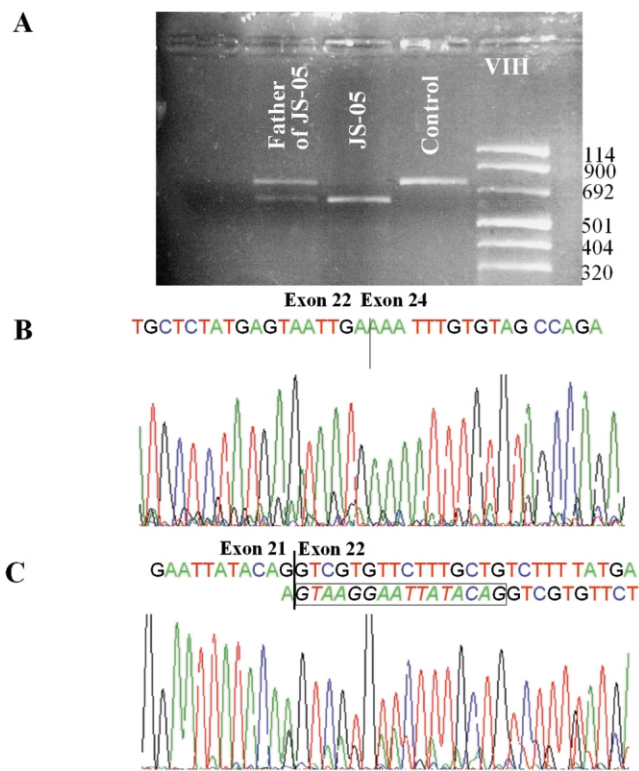
Figure 3. Analysis of two *MKS3* splice-site mutations at the RNA level. *A*, RT-PCR with primers located in *MKS3* exons 20 (forward) and 26 (reverse) in the father of patient JS-05, JS-05, and a control individual. Expected wild-type size (754 bp) is seen in the control individual, whereas in patient JS-05 the smaller band (617 bp) corresponds to the transcript without exon 23. The father of patient JS-05 shows both normal and mutated transcripts. *B*, Direct sequencing of the RT-PCR of patient JS-05 shows a skipping from exons 22 to 24. *C*, Direct sequencing of the RT-PCR of patient JS-09 showing the wild-type (*upper line*) and abnormal (*lower line*) transcripts corresponding to the insertion of 16 intronic bp (*boxed*).

without hydrocephalus, but does not take into account the imaging definition—in particular, on brainstem and high tentorium—that would distinguish among JS, a DW malformation, or a variant. Confusion still exists nowadays, especially for prenatal cases in which MRI was not performed, and many fetuses with a DW malformation diagnosed at neuropathological examination could be affected with JS.

Hepatic involvement has been reported in JS, mainly in the form of congenital hepatic fibrosis,[17,24] but bile-duct proliferation of liver (a consistent feature of MKS[13]) was also described at the postmortem examination of a boy with JS who died at age 12 mo.[25] To support this observation, we describe in this report two fetuses that also had a bile-duct proliferation of liver. It is not clear if the *MKS3* missense and splice-site mutations (table 1) that we have identified are hypomorphic alleles, but such a suggestion would be reasonable given the viability and marked phe-

notypic overlap of the *Wpk* rat, the model animal for MKS, with human JS. The *Wpk* rat carries a missense mutation in *Mks3* and does not develop the full range of CNS malformations that are typical of human MKS.[11]

Allelism between JS and MKS has been suggested elsewhere,[26,27] and whether patients with JS with occipital encephalocele compose a distinct entity or are a part of the MKS phenotypic spectrum has been debated.[28] The very recent identification of *CEP290* mutations in two patients with JS with occipital meningoencephalocele[4] provides support that occipital encephalocele belongs to the spectrum of JS. These results suggest that the rare patients reported to have a long-survival MKS because of an occipital meningocele were more likely to have JS.[26,29,30] The phenotypic overlap between these two disorders is also illustrated by the considerable phenotypic variability observed in *JBTS2*-linked patients with multiorgan involvement, including occipital encephalocele, polydactyly, microphthalmia, and kidney disease.[9,31] In view of the present study, at least one gene, *MKS3*, can lead to both phenotypes. It is possible that the allelic nature of these conditions may be extended to the *MKS2* locus, which has been mapped to chromosome 11q13[32] and may therefore be allelic to *JBTS2*, which has been mapped to 11p12-11q13.3.[9] However, Valente et al. has refined the *JBTS2* interval to the region between *D11S4191* and *D11S1344* at 11p11.2-11q12.1, which would argue against this interpretation.[31]

No *MKS1* mutations were identified in the patients with JS, which suggests that the allelism is specific to the *MKS3* locus. In a recent study, we analyzed *MKS1* and *MKS3* genes in 54 fetuses with MKS and found a phenotype/genotype correlation according to the mutated gene. Whereas the occipital encephalocele was constant in fetuses with *MKS1* mutations, DW malformations were observed in some fetuses with *MKS3*, and polydactyly was significantly less frequent in fetuses with *MKS3* mutations (R.K., unpublished data). The identification of *MKS3* mutations in patients with JS adds to the phenotypic variability associated with *MKS3* mutations, ranging from a severe and lethal cystic kidney dysplasia with exencephalocele to a mild form of MTS with normal kidneys and moderate mental retardation. Since no obvious genotype/phenotype correlation could be observed concerning the type or localization of *MKS3* mutations in patients with MKS or JS, one can hypothesize that oligogenic inheritance and/or modifier genes are responsible for the highly variable phenotypes associated with *MKS3* mutations. *MKS1* is unlikely to be involved, since we did not identify *MKS1* mutations in any patients with the *MKS3* mutation.

Genes involved in NPHP/JS and MKS encode proteins involved in ciliary function, and the conditions are collectively known as "ciliopathies."[33] Little is known about the function of *AHI1*, whereas *NPHP* genes leading to NPHP with (*NPHP1* and *CEP290*[4,34]) or without (*NPHP2*,[35] *NPHP4*,[36] and *NPHP5*[37]) cerebellar vermis hypoplasia encode proteins located at the primary cilia and centro-

somes. In addition, some of these proteins have been shown to interact, suggesting that they belong to the same signaling pathway.[35,36,38] Comparative genomics also suggests that *MKS1* and *MKS3* encode ciliary proteins.[10,11] Bardet-Biedl syndrome (BBS [MIM 209900]) is another multisystemic genetic disorder that has phenotypic overlap with MKS[39] and JS and is also thought to be a ciliopathy.[40] In BBS, the genetic basis of phenotypic variability is provided by oligogenism, since three mutated *BBS* alleles are necessary for the expression of the disease in some families,[41] whereas a third mutated allele modulates the phenotype in other instances.[42] Epistatic interactions between proteins interacting and colocalizing with the BBS proteins were also demonstrated in BBS.[43] Oligogenism is also suggested in NPHP and JS. Indeed, in several patients, only one mutation was identified at *NPHP3*[38] or *CEP290*[4] loci. In our study, a single *MKS3* heterozygous mutation, F29L, was also identified in a fetus with vermis agenesis, a small occipital bone defect, cystic kidney dysplasia, and no bile-duct proliferation of liver. Either we failed to find the second molecular event or this mutation, lying in the putative peptide signal of meckelin, plays an epistatic role, along with mutations in another gene.

The existence of a distinct phenotype/genotype correlation in JS with some rare cases of phenotypic variability argues for such an oligogenic model. Indeed, whereas *AHI1* mutations lead to JS with retinal involvement and sometimes polymicrogyria,[44] *AHI1* mutations were found only in two patients with kidney involvement.[45] Also, *NPHP1* is responsible for isolated NPHP in the vast majority of cases; of patients with JS, only several with a mild form of the brain malformation[6,46] and one with retinal involvement[47] were found with the *NPHP1* deletion. One can hypothesize that oligogenic or epistatic inheritance is responsible for the additional signs found in these rare patients. The probability of such an additional molecular event is high, in view of the large number of genes involved in this group of ciliopathies and retinal dystrophies.[33] In contrast with *AHI1* and *NPHP1,* extensive phenotypic variability is observed in patients with *CEP290* mutations who have both renal and ocular variable involvement and in two patients with occipital encephalocele. *CEP290* mutations were even shown to be a frequent cause of isolated Leber congenital amaurosis.[48] This large variability is also observed in patients with *MKS3* mutations. A phenotype/genotype correlation is also found at *JBTS1* and *JBTS2* loci: whereas no organ involvement is described in *JBTS1*-linked patients, a large variability is associated with *JBTS2*-linked patients with multiorgan involvement, including occipital encephalocele, polydactyly, microphthalmia, and kidney involvement.[31]

The identification of genes leading to vermis agenesis, DW malformation, or occipital encephalocele supports the existence of an embryologic mechanism common to these brain malformations. The encephalocele may be secondary to the brainstem and cerebellum herniation and not to a primary neural tube defect. Indeed, in most patients with MKS who underwent detailed neuropathological examination,[49,50] occipital encephalocele consists of extruded rhombic roof elements and third ventricle and a distended fourth ventricle through a widened posterior fontanelle. However, a second bony defect was frequently observed below the meningocele or meningoencephalocele and cannot be due to the herniation. In addition, a wide spectrum of CNS anomalies are usually observed in MKS, including heterotopia, micropolygyria, optic nerve and chiasma hypoplasia, and midline defects ranging from corpus callosum agenesis to arhinencephaly, hypothalamic fusion, and complete holoprosencephaly. Whether these prosencephalic and rhombencephalic dysgeneses could also be mechanical consequences of the ex-encephalocele is questionable. On the other hand, malformations of pontine and medullary structures, as well as cerebellar neuron heterotopias, are described in patients with JS,[51] and mutations in *AHI1* lead to JS with cortical polymicrogyria,[44] which suggests a more general defect in axon guidance in JS. Primary cilia are found in many brain cell types (reviewed by Fuchs et al.[52]). Whether they act as mechanosensory organelles of the cerebrospinal fluid or are involved in the planar cell polarity pathway[53] or both is still unknown. It has recently been shown that intraflagellar transport proteins are necessary for neuronal cilia biogenesis and for Hedgehog signal transduction,[54] providing a potential molecular explanation for the midline defects/holoprosencephaly that can be observed in MKS.

In conclusion, the identification of *MKS3* gene mutations in four patients with JS defines the *MKS3* gene as the sixth locus for JS (*JBTS6*). Although the malformative spectrum observed in MKS is more severe, the identification of *MKS3* mutations in patients with JS suggests that JS and MKS are allelic disorders and that other and still-unknown molecular defects could be responsible for the variable phenotypic expression of *MKS3* mutations.

Web Resources

The URLs for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM),http://www.ncbi.nlm.nih.gov/Omim/ (for JS, MKS, and BBS)
PolyPhen, http://genetics.bwh.harvard.edu/pph/
Splice Site Prediction by SSF, http://www.umd.be/SSF
TMHMM Server, http://www.cbs.dtu.dk/services/TMHMM/

References

1. Joubert M, Eisenring JJ, Andermann F (1968) Familial dysgenesis of the vermis: a syndrome of hyperventilation, ab-

normal eye movements and retardation. Neurology 18:302–303

2. Patel S, Barkovich AJ (2002) Analysis and classification of cerebellar malformations. AJNR Am J Neuroradiol 23:1074–1087

3. Ferland RJ, Eyaid W, Collura RV, Tully LD, Hill RS, Al-Nouri D, Al-Rumayyan A, Topcu M, Gascon G, Bodell A, et al (2004) Abnormal cerebellar development and axonal decussation due to mutations in *AHI1* in Joubert syndrome. Nat Genet 36:1008–1013

4. Sayer JA, Otto EA, O'Toole JF, Nurnberg G, Kennedy MA, Becker C, Hennies HC, Helou J, Attanasio M, Fausett BV, et al (2006) The centrosomal protein nephrocystin-6 is mutated in Joubert syndrome and activates transcription factor ATF4. Nat Genet 38:674–681

5. Valente EM, Brancati F, Silhavy JL, Castori M, Marsh SE, Barrano G, Bertini E, Boltshauser E, Zaki MS, Abdel-Aleem A, et al (2006) *AHI1* gene mutations cause specific forms of Joubert syndrome-related disorders. Ann Neurol 59:527–534

6. Parisi MA, Bennett CL, Eckert ML, Dobyns WB, Gleeson JG, Shaw DW, McDonald R, Eddy A, Chance PF, Glass IA (2004) The *NPHP1* gene deletion associated with juvenile nephronophthisis is present in a subset of individuals with Joubert syndrome. Am J Hum Genet 75:82–91

7. Saar K, Al-Gazali L, Sztriha L, Rueschendorf F, Nur EKM, Reis A, Bayoumi R (1999) Homozygosity mapping in families with Joubert syndrome identifies a locus on chromosome 9q34.3 and evidence for genetic heterogeneity. Am J Hum Genet 65:1666–1671

8. Valente EM, Salpietro DC, Brancati F, Bertini E, Galluccio T, Tortorella G, Briuglia S, Dallapiccola B (2003) Description, nomenclature, and mapping of a novel cerebello-renal syndrome with the molar tooth malformation. Am J Hum Genet 73:663–670

9. Keeler LC, Marsh SE, Leeflang EP, Woods CG, Sztriha L, Al-Gazali L, Gururaj A, Gleeson JG (2003) Linkage analysis in families with Joubert syndrome plus oculo-renal involvement identifies the CORS2 locus on chromosome 11p12-q13.3. Am J Hum Genet 73:656–662

10. Kyttala M, Tallila J, Salonen R, Kopra O, Kohlschmidt N, Paavola-Sakki P, Peltonen L, Kestila M (2006) *MKS1,* encoding a component of the flagellar apparatus basal body proteome, is mutated in Meckel syndrome. Nat Genet 38:155–157

11. Smith UM, Consugar M, Tee LJ, McKee BM, Maina EN, Whelan S, Morgan NV, Goranson E, Gissen P, Lilliquist S, et al (2006) The transmembrane protein meckelin (*MKS3*) is mutated in Meckel-Gruber syndrome and the wpk rat. Nat Genet 38:191–196

12. Gattone VH 2nd, Tourkow BA, Trambaugh CM, Yu AC, Whelan S, Phillips CL, Harris PC, Peterson RG (2004) Development of multiorgan pathology in the *wpk* rat model of polycystic kidney disease. Anat Rec A Discov Mol Cell Evol Biol 277:384–395

13. Salonen R (1984) The Meckel syndrome: clinicopathological findings in 67 patients. Am J Med Genet 18:671–689

14. Ramensky V, Bork P, Sunyaev S (2002) Human non-synonymous SNPs: server and survey. Nucleic Acids Res 30:3894–3900

15. Romano S, Boddaert N, Desguerre I, Hubert L, Salomon R, Seidenwurm D, Bahi-Buisson N, Nabbout R, Sonigo P, Lyonnet S, et al (2006) Molar tooth sign and superior vermian dysplasia: a radiological, clinical, and genetic study. Neuropediatrics 37:42–45

16. Johnson CA, Gissen P, Sergi C (2003) Molecular pathology and genetics of congenital hepatorenal fibrocystic syndromes. J Med Genet 40:311–319

17. Satran D, Pierpont ME, Dobyns WB (1999) Cerebello-oculo-renal syndromes including Arima, Senior-Loken and COACH syndromes: more than just variants of Joubert syndrome. Am J Med Genet 86:459–469

18. Raynes HR, Shanske A, Goldberg S, Burde R, Rapin I (1999) Joubert syndrome: monozygotic twins with discordant phenotypes. J Child Neurol 14:649–654,669–672

19. Moerman P, Pauwels P, Vandenberghe K, Lauweryns JM, Fryns JP (1993) Goldston syndrome reconsidered. Genet Couns 4:97–102

20. Balci S, Teksen F, Dokmeci F, Cengiz B, Comert RB, Can B, Ozdamar S (2004) Prenatal diagnosis of Meckel-Gruber syndrome and Dandy-Walker malformation in four consecutive affected siblings, with the fourth one being diagnosed prenatally at 22 weeks of gestation. Turk J Pediatr 46:283–288

21. Egger J, Bellman MH, Ross EM, Baraitser M (1982) Joubert-Boltshauser syndrome with polydactyly in siblings. J Neurol Neurosurg Psychiatry 45:737–739

22. Pierquin G, Deroover J, Levi S, Masson T, Hayez-Delatte F, Van Regemorter N (1989) Dandy-Walker malformation with postaxial polydactyly: a new syndrome? Am J Med Genet 33:483–484

23. van Dorp DB, Palan A, Kwee ML, Barth PG, van der Harten JJ (1991) Joubert syndrome: a clinical and pathological description of an affected male and a female fetus from the same sibship. Am J Med Genet 40:100–104

24. Lewis SM, Roberts EA, Marcon MA, Harvey E, Phillips MJ, Chuang SA, Buncic JR, Clarke JT (1994) Joubert syndrome with congenital hepatic fibrosis: an entity in the spectrum of oculo-encephalo-hepato-renal disorders. Am J Med Genet 52:419–426

25. Silverstein DM, Zacharowicz L, Edelman M, Lee SC, Greifer I, Rapin I (1997) Joubert syndrome associated with multicystic kidney disease and hepatic fibrosis. Pediatr Nephrol 11:746–749

26. Schurig V, Bowen P, Harley F, Schiff D (1980) The Meckel syndrome in the Hutterites. Am J Med Genet 5:373–381

27. Casamassima AC, Mamunes P, Gladstone IM Jr, Solomon S, Moncure C (1987) A new syndrome with features of the Smith-Lemli-Opitz and Meckel-Gruber syndromes in a sibship with cerebellar defects. Am J Med Genet 26:321–336

28. Gleeson JG, Keeler LC, Parisi MA, Marsh SE, Chance PF, Glass IA, Graham JM Jr, Maria BL, Barkovich AJ, Dobyns WB (2004) Molar tooth sign of the midbrain-hindbrain junction: occurrence in multiple distinct syndromes. Am J Med Genet A 125:117,125–134

29. Lowry RB, Hill RH, Tischler B (1983) Survival and spectrum of anomalies in the Meckel syndrome. Am J Med Genet 14:417–421

30. Genuardi M, Dionisi-Vici C, Sabetta G, Mignozzi M, Rizzoni G, Cotugno G, Martini Neri ME (1993) Cerebro-reno-digital (Meckel-like) syndrome with Dandy-Walker malformation, cystic kidneys, hepatic fibrosis, and polydactyly. Am J Med Genet 47:50–53

31. Valente EM, Marsh SE, Castori M, Dixon-Salazar T, Bertini E, Al-Gazali L, Messer J, Barbot C, Woods CG, Boltshauser E, et

al (2005) Distinguishing the four genetic causes of Jouberts syndrome-related disorders. Ann Neurol 57:513–519

32. Roume J, Genin E, Cormier-Daire V, Ma HW, Mehaye B, Attie T, Razavi-Encha F, Fallet-Bianco C, Buenerd A, Clerget-Darpoux F, et al (1998) A gene for Meckel syndrome maps to chromosome 11q13. Am J Hum Genet 63:1095–1101

33. Badano JL, Mitsuma N, Beales PL, Katsanis N (2006) The ciliopathies: an emerging class of human genetic disorders. Annu Rev Genomics Hum Genet 7:125–148

34. Valente EM, Silhavy JL, Brancati F, Barrano G, Krishnaswami SR, Castori M, Lancaster MA, Boltshauser E, Boccone L, Al-Gazali L, et al (2006) Mutations in *CEP290,* which encodes a centrosomal protein, cause pleiotropic forms of Joubert syndrome. Nat Genet 38:623–625

35. Otto EA, Schermer B, Obara T, O'Toole JF, Hiller KS, Mueller AM, Ruf RG, Hoefele J, Beekmann F, Landau D, et al (2003) Mutations in *INVS* encoding inversin cause nephronophthisis type 2, linking renal cystic disease to the function of primary cilia and left-right axis determination. Nat Genet 34: 413–420

36. Mollet G, Salomon R, Gribouval O, Silbermann F, Bacq D, Landthaler G, Milford D, Nayir A, Rizzoni G, Antignac C, et al (2002) The gene mutated in juvenile nephronophthisis type 4 encodes a novel protein that interacts with nephrocystin. Nat Genet 32:300–305

37. Otto EA, Loeys B, Khanna H, Hellemans J, Sudbrak R, Fan S, Muerb U, O'Toole JF, Helou J, Attanasio M, et al (2005) Nephrocystin-5, a ciliary IQ domain protein, is mutated in Senior-Loken syndrome and interacts with RPGR and calmodulin. Nat Genet 37:282–288

38. Olbrich H, Fliegauf M, Hoefele J, Kispert A, Otto E, Volz A, Wolf MT, Sasmaz G, Trauer U, Reinhardt R, et al (2003) Mutations in a novel gene, *NPHP3,* cause adolescent nephronophthisis, tapeto-retinal degeneration and hepatic fibrosis. Nat Genet 34:455–459

39. Karmous-Benailly H, Martinovic J, Gubler MC, Sirot Y, Clech L, Ozilou C, Auge J, Brahimi N, Etchevers H, Detrait E, et al (2005) Antenatal presentation of Bardet-Biedl syndrome may mimic Meckel syndrome. Am J Hum Genet 76:493–504

40. Ansley SJ, Badano JL, Blacque OE, Hill J, Hoskins BE, Leitch CC, Kim JC, Ross AJ, Eichers ER, Teslovich TM, et al (2003) Basal body dysfunction is a likely cause of pleiotropic Bardet-Biedl syndrome. Nature 425:628–633

41. Katsanis N, Lupski JR, Beales PL (2001) Exploring the molecular basis of Bardet-Biedl syndrome. Hum Mol Genet 10: 2293–2299

42. Badano JL, Kim JC, Hoskins BE, Lewis RA, Ansley SJ, Cutler DJ, Castellan C, Beales PL, Leroux MR, Katsanis N (2003) Heterozygous mutations in *BBS1, BBS2* and *BBS6* have a potential epistatic effect on Bardet-Biedl patients with two mutations at a second BBS locus. Hum Mol Genet 12:1651–1659

43. Badano JL, Leitch CC, Ansley SJ, May-Simera H, Lawson S, Lewis RA, Beales PL, Dietz HC, Fisher S, Katsanis N (2006) Dissection of epistasis in oligogenic Bardet-Biedl syndrome. Nature 439:326–330

44. Dixon-Salazar T, Silhavy JL, Marsh SE, Louie CM, Scott LC, Gururaj A, Al-Gazali L, Al-Tawari AA, Kayserili H, Sztriha L, et al (2004) Mutations in the *AHI1* gene, encoding jouberin, cause Joubert syndrome with cortical polymicrogyria. Am J Hum Genet 75:979–987

45. Utsch B, Sayer JA, Attanasio M, Pereira RR, Eccles M, Hennies HC, Otto EA, Hildebrandt F (2006) Identification of the first *AHI1* gene mutations in nephronophthisis-associated Joubert syndrome. Pediatr Nephrol 21:32–35

46. Caridi G, Dagnino M, Rossi A, Valente EM, Bertini E, Fazzi E, Emma F, Murer L, Verrina E, Ghiggeri GM (2006) Nephronophthisis type 1 deletion syndrome with neurological symptoms: prevalence and significance of the association. Kidney Int 70:1342–1347

47. Castori M, Valente EM, Donati MA, Salvi S, Fazzi E, Procopio E, Galluccio T, Emma F, Dallapiccola B, Bertini E (2005) NPHP1 gene deletion is a rare cause of Joubert syndrome related disorders. J Med Genet 42:e9

48. den Hollander AI, Koenekoop RK, Yzer S, Lopez I, Arends ML, Voesenek KE, Zonneveld MN, Strom TM, Meitinger T, Brunner HG, et al (2006) Mutations in the *CEP290* (*NPHP6*) gene are a frequent cause of Leber congenital amaurosis. Am J Hum Genet 79:556–561

49. Paetau A, Salonen R, Haltia M (1985) Brain pathology in the Meckel syndrome: a study of 59 cases. Clin Neuropathol 4: 56–62

50. Ahdab-Barmada M, Claassen D (1990) A distinctive triad of malformations of the central nervous system in the Meckel-Gruber syndrome. J Neuropathol Exp Neurol 49:610–620

51. Yachnis AT, Rorke LB (1999) Neuropathology of Joubert syndrome. J Child Neurol 14:655–659,669–672

52. Fuchs JL, Schwark HD (2004) Neuronal primary cilia: a review. Cell Biol Int 28:111–118

53. Ueno N, Greene ND (2003) Planar cell polarity genes and neural tube closure. Birth Defects Res C Embryo Today 69: 318–324

54. Huangfu D, Liu A, Rakeman AS, Murcia NS, Niswander L, Anderson KV (2003) Hedgehog signalling in the mouse requires intraflagellar transport proteins. Nature 426:83–87