



Joubert Syndrome: Insights Into Brain Development, Cilium Biology, and Complex Disease

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Joubert syndrome (JS) is a primarily autosomal recessive condition characterized by hypotonia, ataxia, abnormal eye movements, and intellectual disability with a distinctive mid-hindbrain malformation (the “molar tooth sign”). Variable features include retinal dystrophy, cystic kidney disease, liver fibrosis and polydactyly. Recently, substantial progress has been made in our understanding of the genetic basis of JS, including identification of seven causal genes (*NPHP1*, *AHI1*, *CEP290*, *RPGRIP1L*, *TMEM67/MKS3*, *ARL13B* and *CC2D2A*). Despite this progress, the known genes account for <50% of cases and few strong genotype-phenotype correlations exist in JS; however, genetic testing can be prioritized based on clinical features. While all seven JS genes have been implicated in the function of the primary cilium/basal body organelle (PC/BB), little is known about how the PC/BB is required for brain, kidney, retina and liver development/function, nor how disruption of PC/BB function leads to diseases of these organs. Recent work on the function of the PC/BB indicates that the organelle is required for multiple signaling pathways including sonic hedgehog, WNT and platelet derived growth factor. Due to shared clinical features and underlying molecular pathophysiology, JS is included in the rapidly expanding group of disorders called ciliopathies. The ciliopathies are emerging as models for more complex diseases, where sequence variants in multiple genes contribute to the phenotype expressed in any given patient.

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In 1969, Marie Joubert et al¹ could hardly have predicted the importance of the obscure autosomal recessive disorder, now named Joubert syndrome (JS).² Almost 30 years after the first description of hypotonia, ataxia, abnormal eye movements, and alternating hyperpnea and/or apnea in 4 French Canadian siblings, the “molar tooth sign,” was identified as the key feature on magnetic resonance imaging (MRI).³ After 7 years, the first causal genes were identified (*NPHP1* and *AHI1*),^{4–6} followed quickly by *CEP290*, *RPGRIP1L*, *TMEM67/MKS3*, *ARL13B*, and *CC2D2A*.^{7–11} All of these genes and their gene products have been associated with the primary cilium and basal body (PC/BB), subcellular organelles the central roles of which in diverse cellular processes have only recently been appreciated (for reviews see Yoder, 2008),¹² making JS one of the rapidly expanding group of disorders termed ciliopathies.¹³ In addition to its

importance for understanding the genetic networks underlying normal and abnormal brain development, JS represents a relatively simple model for more common, genetically complex disorders, in which multiple genetic and environmental factors contribute to the phenotype seen in a given patient.

Clinical Features

Many features of JS (mid-hindbrain malformation, retinal dystrophy, cystic renal disease, congenital hepatic fibrosis, and polydactyly) overlap with ciliopathies in general, and with Meckel syndrome (MKS) in particular (Table 1). Recent evidence indicates that these overlapping phenotypic features reflect a shared molecular pathophysiology involving the PC/BB organelle (see “Clinical and genetic overlap with other disorders”).

Brain Imaging

The term molar tooth sign was coined by María et al³ (1997) to refer to the appearance of long, thick superior cerebellar peduncles, a deep interpeduncular fossa and vermis hypoplasia or aplasia in the same axial slice on

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Table 1 Prevalence of Clinical Features in a Cohort of 235 Families With JS. Core Features seen in 100% of Subjects: Hypotonia, Ataxia, Intellectual Disability, MTS/CVH

Feature	% In Our Cohort
Encephalocele	6
Chorioretinal coloboma	19
Retinal dystrophy	30
Cystic kidney disease	23
Liver disease*	18
Polydactyly	19

*Defined as elevated serum transaminases, abnormal imaging or signs of portal hypertension (varices, hepatosplenomegaly, ascites, upper gastrointestinal bleeding).

brain MRI (Figs. 1A and D). When these features are not captured in the same slice, the following imaging features can be useful in confirming a JS diagnosis. Vermis hypoplasia is best assessed on sagittal views (Figs. 1B, E, H, and K); however, this can be difficult when the hemispheres impinge on the midline. The acquisition of thinner slices has made this less of a problem for more recent clinical imaging studies. Vermis size must also be assessed on axial and coronal views, paying particular attention to whether cerebrospinal fluid is present in the midline between the hemispheres (Figs. 1A, C, D, F, and G). The vermis hypoplasia in JS is unlikely to be due to atrophy, as the vermis has been noted to be small on the earliest possible prenatal imaging at 14-16 weeks of gestation (D. Doherty, personal observations). Evaluation of the fourth ventricle on sagittal views is also informative. In patients with JS, the roof of the fourth ventricle is oriented more horizontally than usual, even when the vermis as a whole is not rotated superiorly (Figs. 1B and H). Finally, although not entirely specific for JS, the fourth ventricle opens widely immediately inferior to the tectum, rather than remaining quite narrow for several millimeters (Fig. 1B). When the vermis is completely absent and a large posterior fossa cyst is present, the radiologic diagnosis of JS can be difficult. A variety of additional imaging features have been observed, including agenesis of the corpus callosum (Fig. 1K),^{14,15} encephalocele (Fig. 1I),^{1,16,17} hydrocephalus,^{14,18} posterior fossa cysts (often labeled Dandy-Walker malformation or

Dandy-Walker variant),¹⁹ cerebellar¹⁷ and cortical heterotopias (Fig. 1J), and polymicrogyria.^{5,16}

Brain Pathology

Neuropathology findings have been reported in at least 15 individuals with JS, ranging from 18 weeks of gestation to 31 years of age (Table 2).^{1,3,8,20-30} Lack of standardization limits the conclusions that can be drawn from these reports; nonetheless, several findings have been reported in more than 1 case. In addition to vermis hypoplasia, the most frequent structures noted to be abnormal are the deep cerebellar nuclei, the inferior olivary nuclei, multiple cranial nerve nuclei, and the decussations of the superior cerebellar peduncles and pyramidal tracts. It is not clear from the pathology which abnormalities are primary and which are secondary. Diffusion tensor imaging and tractography are consistent with defects in the decussation of the superior cerebellar peduncles.³¹⁻³³

Eye Findings

Abnormal eye movements are uniformly present in JS, although variable in severity.^{1-3,34-38} The presence of nystagmus, saccades instead of smooth pursuit, and tracking or acquiring targets with head movements rather than eye movements are the predominant clinical signs. Quantitative eye movement recordings demonstrate that the gains (target velocity and/or eye velocity) for smooth pursuit, saccades, optokinetic nystagmus, and vestibulo-ocular reflex are variably reduced.^{35,39,40} Each of these oculomotor deficits reflect abnormalities of specific neural ensembles in the cerebellar vermis (oculomotor, nodulus, uvula), flocculus or paraflocculus, deep cerebellar nuclei, vestibular nuclei, pontine nuclei, and/or inferior olives. Retinal dystrophy (Fig. 2A) with visual loss occurs in a subset of patients with JS. Early onset of the retinal dystrophy shares overlapping clinical features with Leber congenital amaurosis, and preservation of vision despite markedly abnormal electroretinogram findings supports more severe involvement of rod vs cone photoreceptors in some cases.^{34,41} Chorioretinal coloboma (Fig. 2B), identified in 19% of our cohort, is associated with significant visual impairment if the macula or optic nerve is involved. Strabismus and ptosis are additional ocular findings that may be corrected with surgical intervention.

Figure 1 MRI findings in JS. (A-C) Classic Molar tooth sign (A) Axial T2-weighted image of vermis hypoplasia, long, thick superior cerebellar peduncles and deep interpeduncular fossa. (B) Sagittal T1-weighted image of vermis hypoplasia and elevation of the roof of the fourth ventricle. (C) Coronal T2-weighted image of vermis hypoplasia and thick superior cerebellar peduncles. (D-F) "Mild" molar tooth sign. (D) Axial T2-weighted image of mild vermis hypoplasia and long, mildly thick superior cerebellar peduncles. (E) Sagittal T1-weighted image of mild vermis hypoplasia and elevation of the roof of the fourth ventricle. (F) Coronal T2-weighted image of vermis hypoplasia and thick superior cerebellar peduncles. (G-I) Fetal MRI at 22 weeks of gestation (G) Axial single-shot fast-spin-echo (SSFSE) image of vermis hypoplasia and large posterior fossa fluid collection. (H) Sagittal SSFSE image of vermis hypoplasia and abnormal configuration of the roof of the fourth ventricle. (I) Axial SSFSE image of a small encephalocele (arrowhead). (J) Axial T2-weighted image of periventricular nodular heterotopias (black arrows), vermis hypoplasia, and deep sulcus in the left temporal lobe. (K) Sagittal T1-weighted image of agenesis of the corpus callosum (asterisk) and elevation of the roof of the fourth ventricle. Note that the vermis hypoplasia in B, E, and K is somewhat obscured by the hemispheres impinging on the midline. SSFSE.

Cognition and Behavior

Although most reports indicate that patients with JS have substantial cognitive impairment, the range of ability is quite broad.^{25,42,43} A convenience sample of 32 patients aged 1-17 years (mean 6 years) evaluated with the Child Development Inventory displayed a mean developmental age of 19

months.⁴⁴ Developmental and intelligence quotients between 30 and 80 have been reported in patients with JS;^{38,42} however, the substantial motor and speech impairments make it difficult to accurately measure underlying cognitive ability. Similarly, autism has been reported in children with JS,⁴⁵⁻⁴⁷ but the severe oral-motor dyspraxia and oculomotor issues in

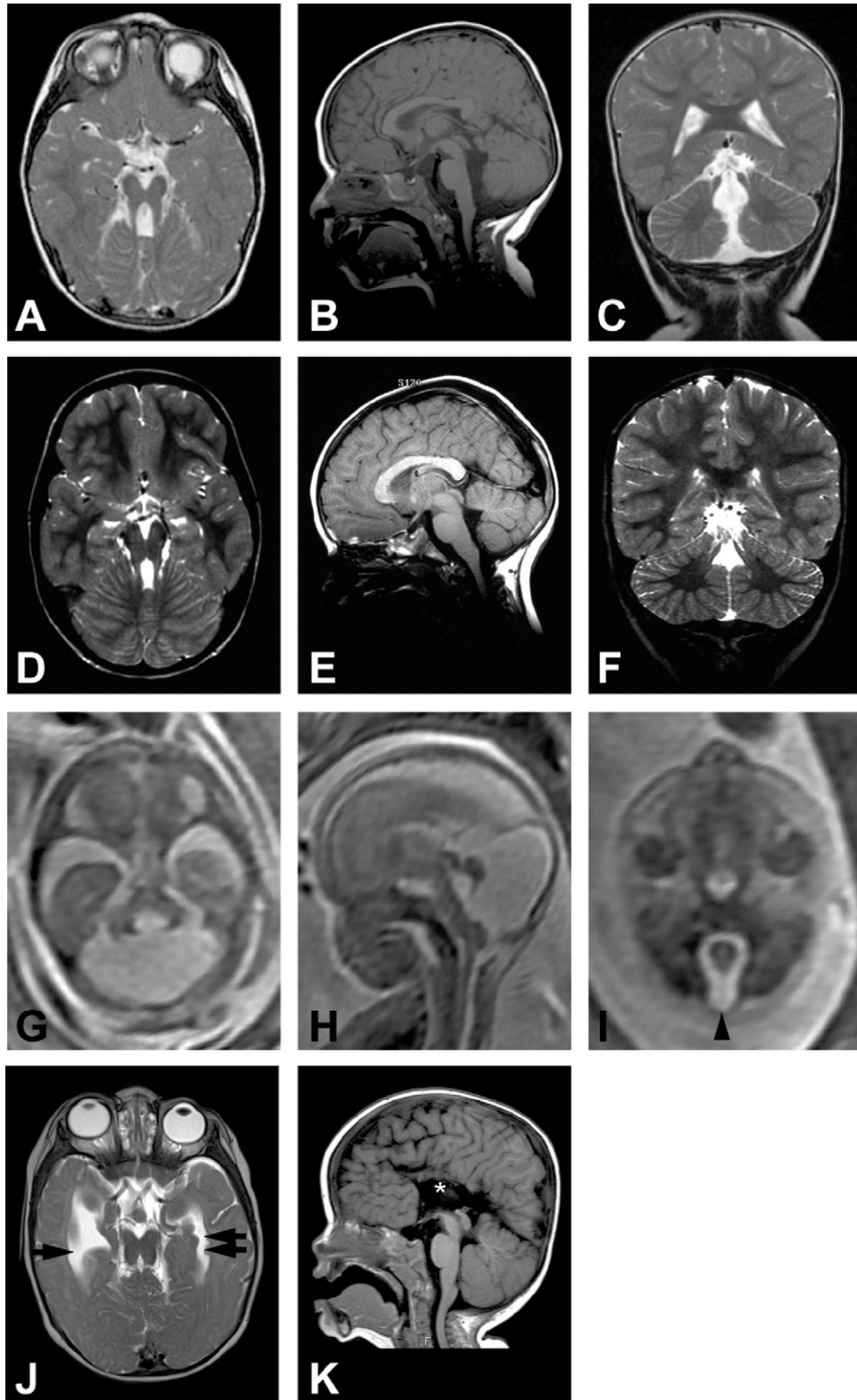


Table 2 Pathologic Brain Abnormalities Reported in Patients With JS

Structure	Pathologic Finding	N*
Cerebellar vermis	Hypoplastic	17/17
Cerebellar flocculus	Abstracts of entomology	2/17
Cerebellar hemispheres	Dysplastic	4/17
Dentate nucleus	Fragmented	10/15
Other deep cerebellar nuclei	Not found	7/15
Superior and inferior colliculi	Fused	2/15
Pyramidal decussation (corticospinal tracts)	Hypoplastic/absent	5/15
Inferior olives	Hypoplastic/dysplastic†	7/15
Superior cerebellar peduncle decussation	Hypoplastic/absent	4/15
Dorsal column nuclei	Hypoplastic/dysplastic	3/15
Cranial nerve nuclei	Hypoplastic/dysplastic	5/15

*Denominators differ because 2 reports did not include histopathology.

†King et al²⁴ (1984) reported hyperplasia of the inferior olives.

JS cause speech delay and abnormal eye contact, complicating the assessment of 2 core features of the diagnosis (communication and social interaction). Takahashi et al⁴⁸ (2006) reported that none of 31 patients with JS exceeded the Autism Behavior Checklist cutoff for autism, and they did not find the increased load of neuropsychiatric issues often seen in families of individuals with autism. In our experience, many patients with JS are interested in social interaction, engage in pretend play, and exhibit theory of mind, which are features that are inconsistent with autism.

Kidney Findings

Similar to the retinal disease seen in JS, the renal disease ranges in severity, from classic nephronophthisis (Fig. 2D) with onset in late childhood or later to cystic renal dysplasia overlapping with MKS (Fig. 2E).^{49,50} The first sign of later onset renal disease is often failure to concentrate urine (salt-wasting renal insufficiency), followed by echogenic kidneys on ultrasound and eventual renal failure. In patients with *AH11* mutations, onset of renal disease has been reported in young adults.^{51,52} Saraiva and Baraitser (1992) reported renal disease in 30% of cases in the published data,¹⁷ similar to the 23% in our cohort.

Liver Disease

COACH syndrome (Cerebellar vermis hypoplasia, Oligophrenia—developmental delay or mental retardation, Ataxia, Colobomas, and Hepatic fibrosis) is considered a subtype of JS.^{16,53,54} Clinically, the liver disease can be asymptomatic or present with mildly elevated serum transaminases, but more often, it is identified by liver imaging or signs of portal hypertension (varices, hepatosplenomegaly, ascites, and rarely, upper gastrointestinal bleeding). Biopsy findings are in the spectrum of the ductal plate malformation

(Fig. 2H) and congenital liver fibrosis (Fig. 2I), and the fibrosis is progressive at least in some cases.⁵⁵ Severe disease requires portosystemic shunting or liver transplantation and can result in death. Liver disease occurs in 18% of our cohort; however, this may be an overestimate of the true prevalence as we have focused on recruiting patients with liver disease.

Skeletal

Polydactyly is seen in 19% of our cohort and is a feature of many ciliopathies. Most frequently, it is postaxial (Fig. 2F), although it can be preaxial or very rarely, mesoaxial. In general, polydactyly is not functionally significant, and surgical correction is at the discretion of the patient or family. As in many children with hypotonia, scoliosis is seen and requires close monitoring, especially during puberty.

Other

Although many patients with JS display dysmorphic facial features, no easily recognizable facial appearance has been described,^{56,57} in contrast to well-known disorders like Down syndrome and Williams syndrome. Oral frenulae, tongue hamartomas, micropenis, and pituitary dysfunction have also been reported in small numbers of patients.^{16,17} Despite the presence of obvious breathing abnormalities in most infants with JS, the prevalence of central and obstructive sleep apnea in older children is likely under appreciated.

Medical Management

Guidelines for the evaluation and management of patients with JS were developed by a consensus panel convened by the Joubert's Syndrome Foundation and Related Cerebellar Disorders, and have been published elsewhere.⁵⁸ These recommendations include yearly ophthalmologic evaluation, urinalysis, renal and liver ultrasounds, as well as serum transaminases, blood urea nitrogen, and creatinine to monitor for and allow early treatment of the medical complications described earlier. Lifelong monitoring for obstructive and central sleep apnea is also warranted. Specific developmental and behavioral supports for JS do not exist, and interventions are tailored to the needs of each individual.

Prevalence

Although the prevalence of JS has been estimated by Flannery and Hudson (1994)⁵⁹ and Parisi and Glass (2007)⁵⁸ to be 1 in 100,000 to 1 in 250,000, no population-based prevalence data exist. On the basis of 30 patients we followed up in our center that draws patients from a region of ~10 million people, a very conservative estimate of the minimum prevalence is ~1 in 300,000.

Genetics of JS and Diagnostic Testing Strategy

Mutations in 7 genes (*NPHP1*, *AH11*, *CEP290*, *RPGRIP1L*, *MKS3/TMEM67*, *CC2D2A*, and *ARL13B*) and 2 additional loci

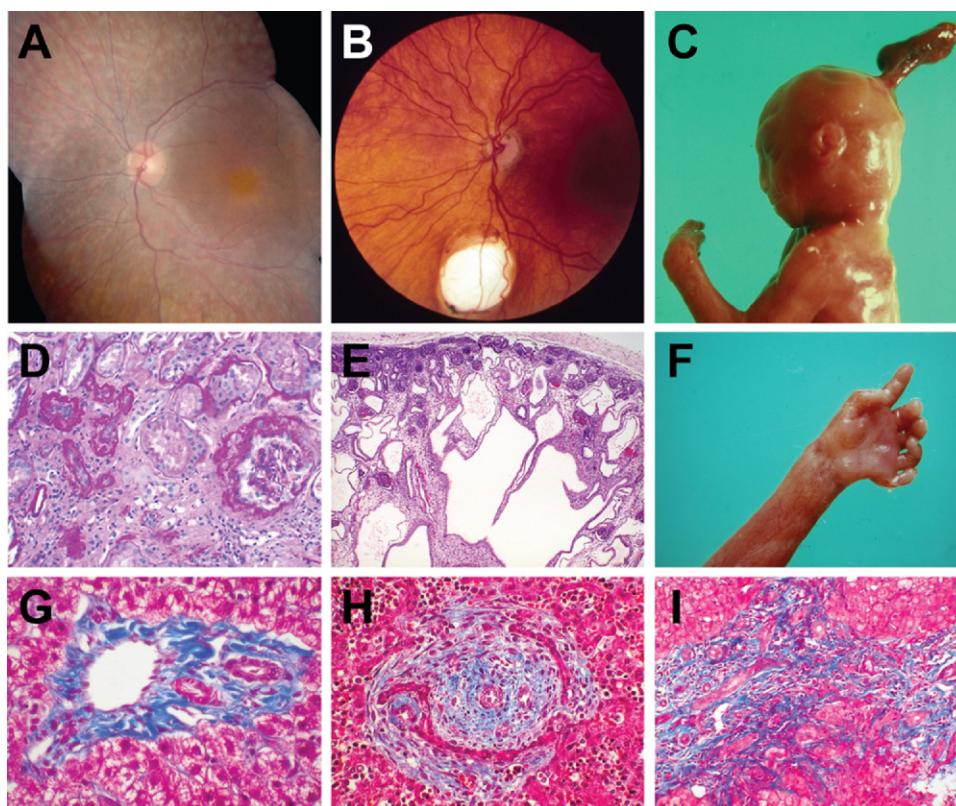


Figure 2 Clinical features in JS (A) Pigmentary retinal dystrophy. (B) Chorioretinal coloboma (pale area). (C) Occipital encephalocele in a 19 weeks of gestation fetus. (D) Nephronophthisis in a mature kidney: interstitial scarring with mild inflammation and tubular atrophy. Basement membranes (dark purple in the online version) around several tubules left and Bowman's capsule right show marked redundancy with wrinkling and lamellation. Periodic acid-Schiff (PAS), 100 \times . (E) Cystic-dysplastic kidney in a 19 weeks of gestation fetus: round, partially collapsed cysts are seen in all parts of the nephron as well as decreased numbers of glomeruli, excessive stroma, and interruption of the nephrogenic zone (upper right, subcapsular). H&E, 40 \times . (F) Postaxial polydactyly in a fetus at 19 weeks of gestation. (G) Normal mature liver: the portal triad contains a branch of the portal vein (left), hepatic artery (middle), and similarly sized bile duct (right) embedded in a small amount of supportive collagen. Trichrome, 200 \times . (H) Ductal plate malformation in a fetus at 22 weeks of gestation: multiple large ducts that parallel the limiting plate and encircle hepatic artery branches are evidence of biliary dysgenesis. Trichrome, 200 \times . (I) Congenital hepatic fibrosis in a 9-year old: the portal triad has moderate fibrosis and an excess of bile ducts and hepatic artery branches with only minimal inflammation. Image courtesy of: Meral Gunay-Aygun, Ekaterini Tsilou (A), Avery Weiss (B), Joseph Siebert (C, F), Laura Finn (D, E, G-I). (Color version of figure is available online.)

(9q34, 11p12-11q13.3) have been associated with JS.^{4-11,60-65} In our cohort, *NPHP1*, *AH11*, *RPGRIPL1*, *ARL13B*, *CC2D2A* and *MKS3/TMEM67* account for <50% of subjects. Due to its large size (54 exons), we have not sequenced *CEP290* in most of our cohort, but it is reported to be responsible for JS in at least 7% of cases.^{11,66}

With the advent of improved sequencing technologies, it will likely become possible to sequence all the JS-related genes simultaneously; however, clinical testing currently involves sequencing each gene individually, so strategies to prioritize testing are important to save time and money. The strongest genotype-phenotype correlation to date is between *MKS3* mutations and clinically apparent liver disease (elevated transaminases, portal hypertension, and/or liver fibrosis on biopsy). Thus, all patients with JS and liver disease should be tested first for *MKS3* mutations. Genotype-phenotype correlations involving the other genes are not as strong (Table 3). For instance, patients with *NPHP1* deletions in-

variably have renal disease and less severe brain malformation. Most subjects with *AH11* mutations have retinal dystrophy, but very few have renal disease. In contrast, *CEP290* mutations cause a spectrum of phenotypes, from isolated JS, to JS with retinal and renal disease, to severe MKS. *RPGRIPL1* mutations also cause a broad spectrum of disease with renal and liver involvement, but only rarely retinal dystrophy. Using these observations, gene sequencing can be prioritized based on the clinical scenario (Fig. 3).

Clinical and Genetic Overlap With Other Disorders: The Ciliopathies

Although similarities between MKS and JS had been noted as early as 1987,⁶⁷ it was not until *CEP290* mutations were found in subjects with JS¹¹ and MKS⁶⁸ that the allelic nature

Table 3 Phenotypic Features and Other Disorders Associated With JS Genes/Loci

Gene/Locus	%*	MTS	Retina	PD	Kidney	Liver	OE	Coloboma	Other Disorders
<i>RPGRIP1L</i>	3	+	±	+	++	+	+	±	MKS
<i>NPHP1</i>	1	±	±	—	++	—	—	—	NPH, Cogan OMA
<i>CEP290</i>	~10	+	++	±	++	+	+	±	MKS, LCA, BBS
<i>MKS3</i>	9	+	—	+	+	++	+	++	MKS
<i>AHI1</i>	8	++	++	—	±	—	—	—	—
<i>ARL13B</i>	<1	+	±	—	—	—	±	—	—
<i>CC2D2A</i>	9	+	±	±	+	±	±	±	MKS
9q34	?	+	±	—	+	—	±	—	?
Chr11	?	+	—	—	+	—	—	±	?
Total	<50								

MTS, molar tooth sign; OE, occipital encephalocele; OMA, oculomotor apraxia; PD, polydactyly; ++, commonly reported; +, reported in some cases; ±, infrequently reported; —, not reported.

*All percentages based on our cohort except for *CEP290* (based on the published data).

of these syndromes was revealed. Since that time, mutations in *RPGRIP1L*, *CC2D2A* and *MKS3/TMEM67* have been shown to cause both MKS and JS.^{7,8,10,14,69,70} Similarly, *NPHP1* mutations can cause isolated nephronophthisis⁷¹ or mild forms of JS.^{4,72} The most striking example is *CEP290*, where mutations can cause JS,¹¹ MKS,^{68,73} and Bardet–Biedl syndrome (BBS),⁷⁴ as well as isolated LCA^{75,76} and nephronophthisis.⁷⁷

This is in keeping with work in human beings and model systems showing that loss of function in genes with related functions cause similar phenotypes (reviewed in Brunner et al⁷⁸ 2004).

As with many disorders, the spectrum of phenotypes associated with JS has expanded because of increased diagnosis in patients with related phenotypes, as well as advances in molecular testing. With the expanded phenotypes has come increasing overlap with other ciliopathies, particularly MKS and BBS, making the clinical classification of individual patients challenging. Not surprisingly in retrospect, these disorders now share molecular causes at least at the level of the genes (if not the specific mutations) involved.

Further complicating matters is the emerging evidence for oligogenic inheritance, whereby full penetrance of a phenotype requires mutations in more than 1 gene. Thus, genetic modifiers may account for the often remarkable variability that is observed in ciliopathies.^{74,79-82} This has been most clearly demonstrated in BBS;⁸¹ however, a higher than expected prevalence of heterozygous *CEP290* and *AHI1* sequence variants was detected in patients with JS caused by mutations in both copies of *NPHP1*.⁸³ In summary, clinical categorizations and causal mutations provide complementary information. Clinical categories remain important for directing diagnostic testing and providing prognostic information, whereas identifying the mutations involved allows for diagnostic, carrier, and prenatal testing, as well as precise recurrence risk counseling.

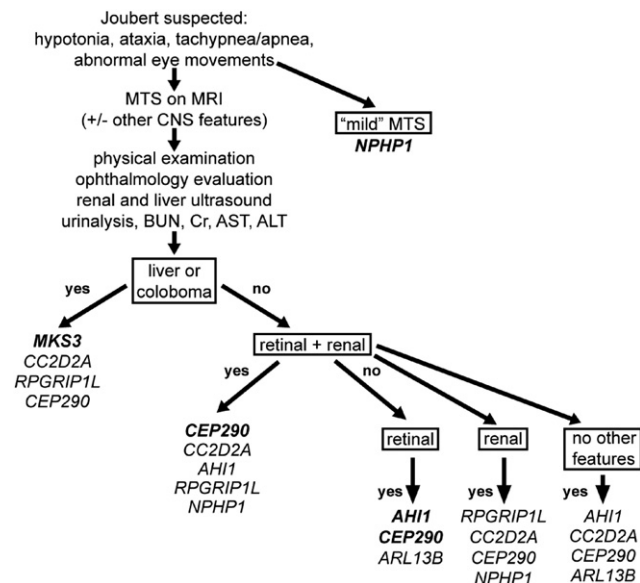


Figure 3 Prioritized genetic testing for JS on the basis of current genotype-phenotype information. Although each of the JS genes can cause a spectrum of phenotypes, patients with a mild MTS, particularly if they have nephronophthisis, should be tested for *NPHP1* deletions and if negative, point mutations. Those with liver disease should be tested for *MKS3/TMEM67* mutations, followed by *CC2D2A* and *RPGRIP1L*. Retinal plus renal disease should prompt testing for *CEP290* mutations. Isolated retinal disease should prompt *AHI1* testing followed by *CEP290*, whereas isolated renal disease should prompt *RPGRIP1L* testing. Patients without organ complications should be tested for *AHI1*, *CC2D2A*, and *CEP290* (particularly the G1890X mutation). If mutations are not found using this strategy, sequencing the remaining genes should be considered. The yield for the currently available DNA testing is predicted to be 40%-50%.

PC/BB and Molecular Mechanisms

Cilia are specialized, membrane-bound, hair-like structures that project from the cell surface (Fig. 4). Each cilium is composed of a microtubule cytoskeleton (the axoneme) surrounded by a specialized membrane and anchored in the cell by the basal body. *Motile cilia* have doublet microtubules arranged in a 9 + 2 concentric pattern and are present on a variety of cell types, including respiratory epithelia, brain ependymal lining cells, and sperm. *Primary cilia* lack the central 2 microtubules (9 + 0 axoneme) and are usually

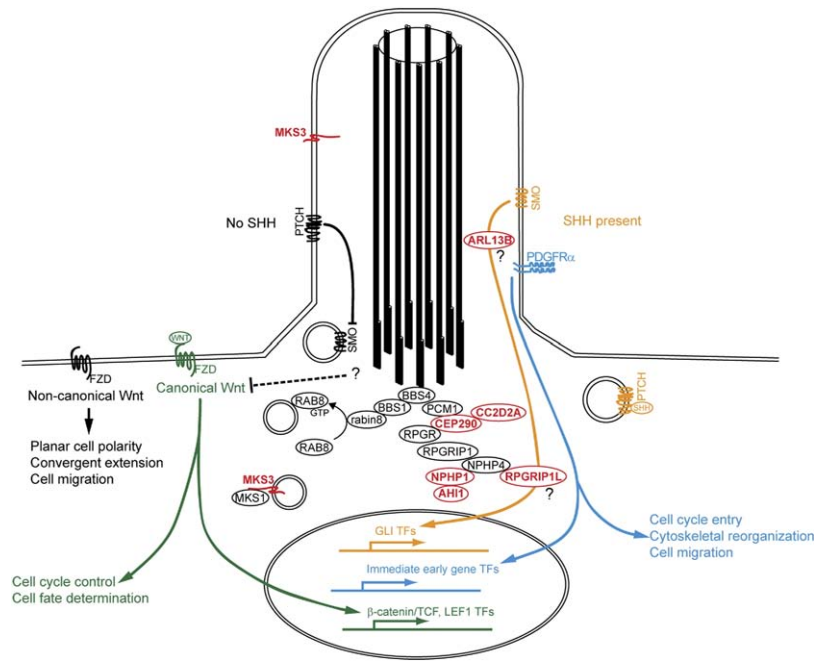


Figure 4 PC/BB, JS gene products, and the SHH, WNT, and PDGF α signaling pathways. The known JS-associated proteins (MKS3, ARL13B, CC2D2A, CEP290, NPH1, AHI1 and RPGRIP1L—red in the online version) have been shown to localize to the PC/BB in at least some contexts. ARL13B, RPGRIP1L, and possibly MKS3, CEP290, and CC2D2A are required for PC/BB formation or maintenance.^{61,69,84,85,86} Direct contact between proteins indicates in vivo or in vitro evidence for physical interactions that are likely cell type and cell cycle-dependent. Activation of the SHH signaling pathway (on right, orange in the online version) relieves the suppression of SMO activity by PTCH, allowing SMO to enter the cilium and alter the balance of GLI activator and repressor transcription factors to control cell fates in a wide variety of tissues (reviewed in Eggenschwiler and Anderson 2007; Wong and Reiter 2008).^{87,88} *ar13b* and *rpgr11* mutations in mice disrupt SHH signaling and cause randomization of left–right asymmetry, neural tube defects, and polydactyly.^{84,85} The PC/BB is required for PDGF α signaling pathway (also on right, blue in the online version) that is involved in regulation of the cell cycle, cytoskeletal organization, and cell migration (reviewed in Andrae et al, 2008; Christensen et al, 2008).^{89,90} Although the mechanism is unknown, the PC/BB also plays a role in regulating the balance between canonical (on left, green in the online version) and noncanonical (also on left, black in the online version) WNT pathways (reviewed in Clevers 2006; Gerdes and Katsanis 2008).^{91,92} SMO, smoothed; TFs, transcription factors. (Color version of figure is available online.)

nonmotile, with the important exception of primitive node cilia that rotate clockwise to generate the leftward nodal fluid flow required for establishing left-right asymmetry in *vertebrates* (reviewed in Shiratori and Hamada 2006 as well as Hirokawa et al, 2006).⁹³⁻⁹⁹

Primary cilia or modifications thereof are present in most cell types, including renal tubule epithelial cells, retinal photoreceptors, chondrocytes, fibroblasts, and neurons. In general, the microtubules that form the axoneme are nucleated by the basal body that forms from the mother centriole when it docks at the plasma membrane after cell division (reviewed in Marshall, 2008).¹⁰⁰ Intraflagellar transport proteins, including dynein and kinesin motors are required for the assembly and function of cilia (reviewed in Pedersen and Rosenbaum).¹⁰¹

Ciliary membranes contain receptors and ion channel proteins mediating mechano- and/or chemosensation and other types of cell signaling. Mounting evidence supports a role for the PC/BB in the sonic hedgehog (SHH), WNT, and platelet-derived growth factor (PDGF α) signaling pathways that control diverse processes, such as cell division, differentiation,

migration, and planar cell polarity. SHH binding to its transmembrane receptor, Patched (PTCH), results in increased localization of smoothed (SMO) to the primary cilium. This binding abolishes the inhibitory effect of PTCH on SMO and transduces signals to the nucleus through the GLI transcription factors, resulting in de-repression and activation of SHH target genes (reviewed in Satir and Christensen 2007).¹⁰² SHH signaling is required for at least 2 major processes in hind-brain development: early for dorsal-ventral patterning of the neural tube, and later for proliferation of the cerebellar granule cells.^{103,104}

In light of the phenotypic overlap between JS and other ciliopathies, it is not surprising that the proteins encoded by all the JS genes have been shown to localize to the PC/BB or directly interact with components of the PC/BB (Table 4).^{7,10,11,14,61,65,69,84,105,106} In addition, mutations in the mouse orthologs of 2 JS genes (*rpgr11* and *ar13b*) disrupt cilium number or morphology and cause aberrant dorsal-ventral patterning of the early neural tube,^{84,85} but the later defects in brain development have not been explored. It is unclear whether the human *RPGRIP1L* and

Table 4 JS Gene Function

Gene	Size (aa)	PC/BB Function	Domains	Animal Mutant
<i>NPHP1</i>	732	BB localization; interaction w/NPHP2-4, AHI1	SH3	M ¹²¹
<i>RPGRIP1L</i>	1315	PC defects; BB and PC localization; interaction with NPHP4	C2, CC, RID	M ⁸⁵
<i>CEP290</i>	2481	BB localization	CC, SMC, more	None
<i>TMEM67/MKS3</i>	995	PC defects; BB and PC localization; interaction with MKS1	7 TM, cysteine rich	M ¹¹⁵ W R ⁷⁰
<i>AHI1</i>	1196	BB localization; interaction with NPHP1	CC, WD40, SH3	None
<i>ARL13B</i>	428	PC defects; PC localization	CC, GTP, proline rich	M ⁸⁴ W Z ⁹
<i>CC2D2A</i>	1620	BB localization; interaction w/CEP290	C2, CC	Z ¹⁴

aa, amino acid; BB, basal body; C2, protein kinase C Ca²⁺ binding domain; CC, coiled-coil; GTP, GTP-binding domain; M, mouse; PC, primary cilium; R, rat; RID, RPGR interacting domain; SH3, src homology domain; SMC, structural maintenance of chromosomes; TM, transmembrane domain; Z, zebrafish.

ARL13B phenotypes are milder because the mutations identified in humans have less severe effects on overall PC/BB function, effects on a subset of PC/BB functions, or effects outside the PC/BB. Disruption of cilium function by loss of *ift88* function after E13 in the mouse cerebellum results in markedly decreased granule cell proliferation without disruption in overall patterning of the cerebellum or brainstem.^{107,108} Purkinje cells are also abnormal, potentially secondary to the granule cell defect. Conditional loss of *kif3a* function generates a similar phenotype, supporting a specific role for the cilium. Given the requirement for intraflagellar transport proteins in granule cell proliferation, it would not be surprising if *RPGRIP1L* and *ARL13B* were also required for granule cell proliferation, likely through effects on SHH signaling.

The JS genes are highly conserved across all *vertebrates*, with orthologs in additional species as distantly related as sea urchin, *nematodes*, and insects. *NPHP1* encodes nephrocystin-1 that interacts with AHI1, other NPHP proteins, and components of cell-cell and cell-matrix signaling pathways. Nephrocystin-1 is localized to the transition zone of the PC/BB in renal epithelia and also to the adherens junctions and focal adhesions in a cell cycle-dependent manner (reviewed in Hildebrandt and Zhou, 2007).¹⁰⁹

RPGRIP1L encodes the *RPGRIP1L* protein which, like its retinally expressed homolog *RPGRIP*, interacts with the *NPHP4* gene product, nephrocystin-4, a ciliary protein defective in some cases of isolated nephronophthisis and Senior-Løken syndrome (nephronophthisis plus retinal dystrophy/LCA).⁷ Mutations in both *RPGRIP1L* and *NPHP4* disrupt this interaction. *RPGRIP1L* localizes to the basal body and also colocalizes with *CEP290* in brain and kidney. Knock out of the murine ortholog of *RPGRIP1L* results in ciliary assembly or functional defects, abnormal left-right asymmetry and pre-axial polydactyly, all likely resulting from disturbed *Shh* signaling.⁸⁵

The protein encoded by *CEP290* localizes to centrosomes and the mitotic spindle in a cell cycle-dependent manner and has been shown to directly activate the activating transcription factor 4 transcription factor in cultured cells.^{11,110} A homozygous in-frame deletion found in a murine retinal dys-

plasia model (*rd16*) abolished interaction with *RPGR*, a microtubule transport protein, suggesting a specific role for *CEP290* in microtubule-based ciliary transport.¹¹¹ Recently, *CEP290* has been implicated in vesicle transport and cilium formation or maintenance through its interactions with *PCMI*¹¹² and *CP110*,¹¹³ as well as its role in transport of G-proteins into olfactory cilia.¹¹⁴

TMEM67/MKS3 encodes the protein meckelin (*MKS3*) that localizes to the primary cilium and to the plasma membrane. *MKS3* interacts with *MKS1* and knockdown of either *MKS1* or *MKS3* by siRNA in ciliated renal epithelia blocked migration of the basal body to the plasma membrane and primary cilium formation.⁶¹ In contrast to these results, cilia are present, albeit elongated and dysmorphic, in a recently published study on *mks3* knockout mouse.¹¹⁵

AHI1 encodes a protein with WD40 and SH3 domains and is highly expressed in human fetal brain and kidney. In post-natal mouse brain, expression is detected at higher levels in the cerebellar dentate nuclei and the deep cortical neurons that form the corticospinal tract, potentially correlating with the failure of decussation observed in functional and neuropathologic studies of JS.^{5,6} *AHI1* localizes to the cell junctions and centrosomes in cultured cells and interacts with *NPHP1* and *HAP1*.^{65,116}

ARL13B is an ADP ribosylation factor (Arf)-related gene in the Ras superfamily of small GTPases, some of which have been implicated in cytoskeletal dynamics, lipid metabolism, and vesicle trafficking. *ARL13B* localizes to cilia in the mouse node, neural tube and fibroblasts, and an *arl13b* null mutation in mice causes neural tube defects, polydactyly, and aberrant *Shh* signaling in the neural tube.^{84,117} Loss of function in the zebrafish *scorpion* mutant results in abnormal body shape and pronephric (kidney) cysts.¹¹⁸

CC2D2A encodes a protein with similar overall structure to *RPGRIP1L*, including coiled-coil domains, a C2 domain, and an overlapping centrosomal protein-related domain. *CC2D2A* physically interacts with *CEP290*, and loss of *Cc2d2a* function in the zebrafish *sentinel* mutant results in abnormal body shape and pronephric (kidney) cysts that is strongly exacerbated by knockdown of *Cep290* function.¹⁴

Recently, the BBS proteins have been shown to form a

complex (the BBSome) that associates with PCM1 and rab8, and is required for sorting proteins to the cilium.¹¹⁹ CEP290 has been shown to be part of the complex and is required for transporting olfactory receptors to the olfactory cilium.^{112,114,107} Other BBS proteins share homology with chaperonins and are likely required to help build the BBSome complex (reviewed in Tobin and Beales, 2007).¹²⁰ The JS gene products share protein domains with BBS gene products, including coiled coils (RPGRIP1L, CC2D2A, CEP290, ARL13B, and BBS2, 4, 7, 9), WD40 repeats (AHI1 and BBS1, 2, 7), and Rab GTPase motifs (ARL13B and ARL6), so it is tempting to hypothesize that the JS proteins also function as a multiprotein complex with members that play similar roles to the BBS proteins (Table 4).

Despite remarkable advances in the genetics of JS, little is known about how specific gene defects result in abnormal brain development. A few hypotheses can be generated on the basis of the limited data available. As described earlier, the brain malformation in JS comprises at least several components: (1) decreased vermis size (likely due to decreased cell numbers); (2) aberrant axonal path finding (disrupted decussation of the superior cerebellar peduncles and pyramids); and (3) possible abnormal neuronal migration (fragmented dentate nuclei, cerebellar and cortical heterotopias, "pachygyric" inferior olives).

One theory proposes that the vermis hypoplasia in JS is due to decreased granule cell proliferation caused by aberrant SHH signaling through defective cilia; however, conditional knock out of *kif3a* and *ift88* in the developing mouse cerebellum results in markedly decreased size of the cerebellar hemispheres and vermis,^{107,108} so somehow, mutations that cause JS would have to affect the vermis preferentially. Alternatively, defective dorsal-ventral patterning could result in alterations to the cells fated to become the cerebellar vermis and deep cerebellar nuclei. To date, defective SHH signaling in the developing cerebellum has not been reported in the existing mouse models for JS. An alternative theory is that subtle alterations in specification of the mid-hindbrain boundary and resulting rhombomere identities could underlie JS. SHH signaling^{122,123} and cilium function¹²⁴ are required for normal specification of the mid-hindbrain boundary, and altered segment identity could affect the birth, cell fate, persistence, or migration of cerebellar cell types. Consistent with this theory is the observation that the vermis develops from the most rostral portion of rhombomere 1 after morphogenetic movements transform the rostral-caudal axis into a medial-lateral orientation.¹²⁵ Tissue-specific elimination of JS gene function at various stages of mid-hindbrain development may clarify the mechanisms, but given the complexity of PC/BB function, it is likely that none of these theories will fully explain the mid-hindbrain phenotype in JS.

Future Directions

Despite great progress in the understanding of JS, many questions remain unanswered. Clinically, prenatal diagnosis remains an issue for >50% of families in whom the genetic cause has not been identified. Prognostic information in the

literature is limited by small numbers of patients, diverse ascertainment strategies, short duration of follow-up, and lack of standardized assessments. We do not yet have accurate information on the easiest outcomes to measure: lifespan and cause of death. The neuropathologic findings in human beings and model organisms are incompletely characterized, and while the known genes are implicated in the PC/BB, the precise molecular function of these genes remains elusive, and many of the players have yet to be identified. Furthermore, the details of how PC/BB dysfunction results in brain malformation and organ dysfunction in JS remains to be explored.

Technological breakthroughs may help with some of these issues (eg, resequencing many genes to identify genetic modifiers that more fully explain JS phenotypes), whereas other issues will require time-consuming, detail-oriented patient phenotyping. Autopsies (pre- and postnatal) have the potential to reveal the most information about the human brain malformation in JS and should be performed by experienced neuropathologists whenever possible. Finally, high-throughput technologies to evaluate protein-protein interaction networks combined with genetic approaches in model systems to validate the protein-protein interactions will yield a more accurate view of the genetic and protein networks underlying JS, as well as normal brain development.

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