Joubert syndrome
Joubert syndrome (JBTS) is characterized by hypotonia, oculomotor apraxia, nystagmus, and intellectual disability. In these patients, brain MRI reveals the pathognomonic “molar tooth sign” (MTS) with absent or hypoplastic cerebellar vermis, deepened interpeduncular fossa, and elongated superior cerebellar peduncles. The term Joubert syndrome and related disorders (JSRD) is used to describe individuals who, in addition to having the core neurological features, also have additional findings including retinal dystrophy, ocular colobomas, kidney disease, liver fibrosis, occipital encephalocele, oral hamartomas, endocrine abnormalities and polydactyly (1).

Meckel Gruber syndrome
Meckel Gruber syndrome (MKS) is the most common syndromic form of neural tube defect and the classic triad of clinical features is characterized by occipital encephalocele, cystic kidneys and fibrotic changes to the liver. The clinical phenotype has since been broadened to include features such as postaxial polydactyly, skeletal dysplasia, microphthalmia, genital anomalies, cleft lip and palate, and heart defects (2).

The genes implicated in JSRD and MKS all play roles in the formation or function of sensory cilia. Primary cilia are essential for vertebrate development, and mutations affecting this organelle underlie a large group of human malformation syndromes, the ciliopathies. To date, mutations in the Joubert/Meckel-Gruber Syndrome Panel account for approximately 50% of Joubert syndrome cases (1). Mutations in the Meckel Gruber Syndrome Panel account for approximately 75% of cases of Meckel Gruber syndrome (2).

Our Joubert/Meckel-Gruber Syndrome Panel includes all 33 genes listed below. Our Meckel-Gruber Sequencing Syndrome Panel includes all 15 genes listed below. Deletion/duplication analysis is also available for the genes listed below in bold.

### Genes Associated with Joubert Syndrome

<table>
<thead>
<tr>
<th>Genes</th>
<th>Associated Disorder(s)</th>
<th>Clinical Features / Molecular Pathology</th>
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<tbody>
<tr>
<td>AHI1</td>
<td>JBTS3</td>
<td>Parisi et al, 2006 identified mutations in AHI1 in 13/117 patients with JSRD (3). Valenti et al, 2006, identified AHI1 mutations in 7.3% of their patient cohort (4). Nonsense, frameshift, missense and splice site pathogenic sequences have all been described. Retinal dystrophy is a common occurrence in individuals with AHI1 mutations (5). AHI1 codes for a component of a protein complex in the basal body, a ring-like structure that functions in the transition zone at the base of cilia. This complex acts as a barrier to restrict protein diffusion between plasma and ciliary membranes.</td>
</tr>
<tr>
<td>ARL13B</td>
<td>JBTS8</td>
<td>Mutations in ARL13B account for less than 1% of patients with JSRD and the majority of mutations identified in ARL13B to date are missense (6). The ARL13B gene, encoding ADP-ribosylation factor-like protein 13B, is a small GTPase belonging to the class of Arf/Arf family within the Ras superfamily of small GTPases involved in diverse cellular functions.</td>
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### Genes Associated with Meckel Gruber Syndrome

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<tr>
<td>B9D1</td>
<td>MKS9</td>
<td>In a fetus with MKS, Hopp et al (2011) identified a patient who was compound heterozygous for a splice-donor site change in B9D1 and a 1.7 Mb de novo deletion encompassing the other allele (7). The fetus inherited an additional likely pathogenic missense change in CEP290 from its mother, suggesting possible oligogenic inheritance in this disorder.</td>
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<tr>
<td>B9D2</td>
<td>MKS10</td>
<td>In two fetuses with MKS from a consanguineous family, Dowdle et al (2011) identified a homozygous missense mutation in B9D2 (8). Mouse mutations in B9d2 compromise ciliogenesis and result in phenotypes consistent with MKS.</td>
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<tr>
<td>C5orf42</td>
<td>JBTS17</td>
<td>Srour et al, 2012 identified mutations in C5orf42 in 7 French Canadian families with JSRD (9). Nonsense, splice site and missense mutations have been identified in C5orf42. The C5orf42 protein features a transmembrane protein and is expressed widely in the brain.</td>
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<tr>
<td>CC2D2A</td>
<td>JBTS9, MKS6</td>
<td>Mutations in CC2D2A represent a cause of both JSRD and MKS and are responsible for approximately 10% of each disorder (10). Like AH11, CC2DA codes for a component of a protein complex in the basal body, a ring-like structure that functions in the transition zone at the base of cilia. This complex acts as a barrier to restrict protein diffusion between plasma and ciliary membranes.</td>
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<tr>
<td>CEP104</td>
<td>JBTS</td>
<td>Biallelic mutations in CEP104 have been indentified in three unrelated patients with a neurologic form of Joubert Syndrome. Their clinical features include molar tooth sign on MRI, oculomotor apraxia and developmental delay with absence of polidactyly, retinopathy and nephropathy (11). The CEP104 protein localizes on the distal end of the primary cilium during ciliogenesis, and may be required for ciliogenesis and maintaining structural integrity at the ciliary tip (OMIM# 616690).</td>
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<tr>
<td>CEP120</td>
<td>JBTS</td>
<td>Roosing et al.(2016) identified nine distinct biallelic mutations in the CEP120 gene in four patients with Joubert Syndrome, and two fetuses with overlapping ciliopathy phenotypes. All patients with Joubert Syndrome presented with a neurological phenotype consisting of hypotonia, developmental delay and cognitive impairment with no retinal, kidney, liver or skeletal involvement (12). Homozygous mutations in CEP120 have also been reported in short-rib thoracic dysplasia with or without polydactyly (OMIM# 613446).</td>
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<tr>
<td>CEP41</td>
<td>JBTS15</td>
<td>Lee et al, 2012 identified three different homozygous splicing mutations in the CEP41 gene in affected members of three consanguinous (Egyptian and Portuguese) families with JSRD (13). Heterozygous missense changes in CEP41 have also been reported in several ciliopathies including MKS and Bardet Biedl syndrome.</td>
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<tr>
<td>CEP290</td>
<td>JBTS5, MKS4</td>
<td>Mutations in CEP290 account for approximately 20% of patients with JSRD and in a handful of families with MKS and Meckel-like syndrome (14). The vast majority of mutations in CEP290 are truncating. Mutations in CEP290 have also been identified in individuals with Leber Congenital Amaurosis, Bardet-Biedl syndrome and Senior-Loken syndrome. The CEP290 gene encodes a centrosomal protein involved in ciliary assembly and ciliary trafficking.</td>
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<tr>
<td>CSPP1</td>
<td>JBTS21, MKS</td>
<td>In 19 patients from 15 families with JSRD, Tuz et al. (2014) identified biallelic truncating mutations in CSPP1 (15). Among these patients, there was a broad spectrum of phenotypic severity, including four of these individuals who also had features of Jeune asphyxiating thoracic dystrophy. Shaheen, et al (2014) further expanded the phenotypic spectrum with a report of a consanguineous family in which two affected siblings had features of Meckel-Gruber syndrome due to a homozygous truncating mutation in CSPP1 (16). Zebrafish knockouts of cspp1 were consistent with the phenotype observed in other JSRD and resulted in reduced ciliary localization, further supporting the loss of CSPP1 as a cause of ciliopathies (15).</td>
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<tr>
<td>IFT172</td>
<td>JBTS</td>
<td>Halbritter, et al. (2013) identified homozygous or compound heterozygous mutations in 12 families affected by the skeletal ciliopathies asphyxiating thoracic dystrophy (ATD), Sensenbrenner syndrome, and Mainzer-Saldino syndrome (MZSDS). Affected individuals exhibited abnormalities of the thorax and/or long bones and additionally had renal, hepatic, or retinal involvement. In two of the families studies, individuals additionally had cerebellar hypoplasia or aplasia, consistent with co-occurrence of Joubert syndrome with the skeletal ciliopathy (17).</td>
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<tr>
<td>INPP5E</td>
<td>JBTS</td>
<td>Bielas et al. (2009) identified 5 different homozygous missense mutations in INPP5E in 7 consanguineous families from the United Arab Emirates, Turkey, Egypt and Italy (18). Mutations in INPP5E that cause JSRD are typically missense changes. The INPP5E protein cleaves the 5-position phosphate from soluble inositol phosphates or inositol phospholipids and is localized predominantly to the primary cilium.</td>
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<tr>
<td>KIAA0556</td>
<td>JBTS</td>
<td>Sanders et al (2015) identified a novel homozygous truncating variant in KIAA0556 in three siblings in a consanguineous Saudi Arabian family. The three children suffered from developmental delay (two with pituitary abnormalities) and were suspected to have a diagnosis of Joubert syndrome based on neuroimaging studies. Mouse studies by the same group showed that KIAA0556 deficiency results in hydrocephalus, and is consistent with the relatively mild phenotype observed in the affected siblings. In addition, studies in C. elegans support a ciliary function for KIAA0556 (19). Subsequent studies by Roosing et al (2016) identified another consanguineous family in which two siblings with a clinical diagnosis of Joubert syndrome were found to have homozygous mutations in KIAA0556 (20).</td>
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</table>
TMEM67   JBTS23  Roosing et al. (2015) identified homozygous or compound heterozygous mutations in TMEM67 in 6/145 patients with unexplained Joubert syndrome (21). The majority of reported mutations were protein-truncating. Bachmann-Gagescu et al. (2015) found biallelic mutations in TMEM67 in approximately 2.5% of patients from a large cohort of individuals affected with Joubert syndrome. All individuals with TMEM67 mutations were reportedly at the mild end of the Joubert syndrome spectrum (22).

KIF7   JBTS12  Truncating mutations in KIF7 have been identified in a handful of patients with JBTS (23). The relative contribution of mutations in this gene to JSRD remains unknown, as a limited number of individuals with mutations have been described. The KIF7 gene encodes a cilia-associated protein belonging to the kinesin family that plays a role in the hedgehog signaling pathway.

KIF14   MKS12  Filges et al. (2014) identified compound heterozygous truncating mutations in KIF14 in two sibling fetuses with a lethal fetal congenital anomaly syndrome consistent with Meckel Gruber syndrome. Findings included oligohydramnios, intrauterine growth retardation, severe microcephaly, renal agenesis/hypoplasia, complex brain malformations, and genitourinary malformations (24).

OFD1   JBTS10, OFD1  To date, three families with X-linked Joubert syndrome have been identified with truncating mutations in OFD1 (25). Mutations in OFD1 are also implicated in Oral-facial-digital syndrome type 1, a disorder whose features include midline oral clefts (lip, palate and tongue), nodules (hamartomas) on the tongue, and digital abnormalities, including brachydactyly, syndactyly and polydactyly.

MKS1   MKS1  Mutations in MKS1 account for approximately 10% of patients with MKS (2). Truncating, frameshift and splice site mutations have been reported. Missense mutations in MKS1 do not appear to be causative for MKS (2). MKS1 is widely expressed in the brain, liver, kidney and developing digits.

NPHP1   JBTS4  Parisi et al, identified homozygous and compound heterozygous deletions of the NPHP1 gene in individuals with mild JBTS (3, 26). The most consistent clinical findings are development of nephronophthisis with or without retinal dystrophy, milder developmental delay, and a distinctive molar tooth sign on radiologic imaging. Deletions of NPHP1 are also responsible for 20-30% of the purely renal forms of juvenile nephronophthisis and only about 1-3% of individuals with JSRD (5).

NPHP3   MKS7  In four families in which a total of seven individuals had features consistent with MKS, Bergmann et al (2008) identified homozygous or compound heterozygous mutations in NPHP3 (27). Splice-site, nonsense, and missense mutations were observed. Mutations in NPHP3 are also responsible for approximately 1% of juvenile nephronophthisis (28).

PDE6D   JBTS22  In a consanguineous family in which three of five siblings were affected with JBTS, Thomas et al (2014) identified a homozygous splice-site mutation leading to an in-frame deletion of PDE6D exon 3 (29). Mutations were not identified in an additional 940 patients with clinically diagnosed ciliopathy conditions, indicating that this is a rare cause of JBTS. Pde6d knockdown in zebrafish was shown to alter both eye and kidney development (29).

RPGRIP1L   JBTS7, MKS5  Mutations in RPGRIP1L account for approximately 2-4% of patients with JSRD and the phenotypic spectrum includes predominantly renal disease (5). Delouis et al., 2007 identified homozygous and compound heterozygous truncating mutations in 3 fetuses with MKS (30). Mutations in RPGRIP1L that cause MKS tend to be protein-truncating and have a more severe effect on protein function.

TCTN1   JBTS13  Garcia-Gonzalo et al. 2011 identified a homozygous splice site mutation in TCTN1 in a consanguineous family from Bangladesh (31). The relative contribution of mutations in this gene to JSRD remains unknown, as a limited number of individuals with mutations have been described.

TCTN2   MKS8  A homozygous splice site mutation in TCTN2 was identified by Shaheen et al. (2011) in a consanguineous Saudi family with 5 pregnancies affected with MKS (32). Tctn2-null mice lack nodal cilia and have a minimal amount of cilia in the neural tube (31).

TCTN3   JBTS18, OFD4, MKS  Thomas et al. (2012) identified a homozygous mutation in TCTN3 in a consanguineous Turkish family with two siblings with Joubert syndrome (33). Homozygous or compound heterozygous mutations in TCTN3 have also been associated with oralfaciodigital syndrome type 4 (OFD4), which has a high degree of clinical overlap with Meckel Gruber syndrome (33). Functional studies of TCTN3 have found that it is necessary for transduction of the SHH signaling pathway.

TMEM67   JBTS6, MKS3  Doherty et al, 2009, identified mutations in TMEM67 in 19/232 (8%) of individuals with JSRD (34). Mutations in TMEM67 also account for approximately 12% of patients with MKS (2). Mutations identified in MKS rarely overlap with the mutations seen in JSRD and it has been hypothesized that more severe loss of function mutations are more likely to cause MKS, while milder mutations are more associated with JSRD (5).
<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
<th>Details</th>
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<tbody>
<tr>
<td>TMEM107</td>
<td>JBTS, MKS13</td>
<td>Shaheen et al. (2015) identified a homozygous truncating mutation in TMEM107 in two consanguineous families with a history of a pregnancy with a clinical diagnosis of Meckel Gruber syndrome (35). Additionally, Lambacher et al. (2016) identified a compound heterozygous mutation in TMEM107 in a patient with features of Joubert syndrome (36). Fibroblasts from this patient exhibited reduced ciliation and the cilia that formed were abnormally long (36).</td>
</tr>
<tr>
<td>TMEM138</td>
<td>JBTS16</td>
<td>Lee et al. 2012 identified missense and splicing mutations in TMEM138 in 6 consanguineous Arab families with JSRD (37). The phenotype included the molar tooth sign on brain imaging, oculomotor apraxia, variable coloboma, and rare kidney involvement and was indistinguishable from that caused by mutation in TMEM216 gene.</td>
</tr>
<tr>
<td>TMEM216</td>
<td>JBTS2, MKS2</td>
<td>Valente et al. 2010 identified mutations in TMEM216 in 14/462 (14%) of patients with JSRD (38). A common founder mutation (p.R12L) has been identified in the Ashkenazi Jewish population with a carrier rate of 1 in 92 (39). Mutations in TMEM216 also account for less than 5% of patients with MKS (2).</td>
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<tr>
<td>TMEM231</td>
<td>JBTS20, MKS11</td>
<td>Srour et al. (2012) identified compound heterozygous mutations in TMEM231 in 3 individuals with Joubert syndrome from two unrelated French Canadian families (40). Knockout Tmem231 mice show embryonic lethality with microphthalmia, polydactyly and patterning defects of the ventral spinal cord, consistent with a ciliopathy (41). Shaheen et al. (2013) identified homozygous mutations in TMEM231 in two consanguineous families with classic Meckel-Gruber syndrome (42).</td>
</tr>
<tr>
<td>TMEM237</td>
<td>JBST14</td>
<td>Huang et al. 2011 identified homozygous truncating mutations in the TMEM237 in 10 related Canadian Hutterite families with JSRD (43). Screening of an additional 201 individuals with JSRD and 90 individuals with MKS identified two additional patients with mutations in TMEM237.</td>
</tr>
<tr>
<td>TTC21B</td>
<td>JATD, NPHP, JSRD</td>
<td>Davis et al. 2011 sequenced TTC21B in 753 patients with some form of ciliopathy and identified homozygous or compound heterozygous mutations in one Jeune asphyxiating thoracic dystrophy (JATD) pedigree and five nephronophthisis (NPHP) pedigrees (44). In addition, heterozygous likely pathogenic mutations were identified in an additional 38 unrelated cases (5%), and one third of these had a second pathogenic mutation identified in a different ciliopathy gene. Individuals with Joubert syndrome related disorders (JSRD) and a single heterozygous mutation have been reported, although the functional significance of a single mutation is not clear.</td>
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</table>

**Test methods:**

Comprehensive sequence coverage of the coding regions and splice junctions of all genes in this panel is performed. Targets of interest are enriched and prepared for sequencing using the Agilent SureSelect system. Sequencing is performed using Illumina technology and reads are aligned to the reference sequence. Variants are identified and evaluated using a custom collection of bioinformatic tools and comprehensively interpreted by our team of directors and genetic counselors. All novel and/or potentially pathogenic variants are confirmed by Sanger sequencing. The technical sensitivity of this test is estimated to be >99% for single nucleotide changes and insertions and deletions of less than 20 bp. Deletion/duplication analysis of the panel genes is performed by oligonucleotide array-CGH. Partial exonic copy number changes and rearrangements of less than 400 bp may not be detected by array-CGH. Array-CGH will not detect low-level mosaicism, balanced translocations, inversions, or point mutations that may be responsible for the clinical phenotype. The sensitivity of this assay may be reduced when DNA is extracted by an outside laboratory.

**Joubert/Meckel-Gruber Syndrome Sequencing Panel (33 genes sequence analysis)**

- Sample specifications: 3 to 10 cc of blood in a purple top (EDTA) tube
- Cost: $3000
- CPT codes: 81407
- Turn-around time: 8 weeks

**Note:** We cannot bill insurance for the above test.

**Joubert/Meckel-Gruber Deletion/Duplication Panel (26 genes deletion/duplication analysis)**

- Sample specifications: 3 to 10 cc of blood in a purple top (EDTA) tube
- Cost: $2500
- CPT codes: 81407
- Turn-around time: 6 weeks

**Note:** The sensitivity of our assay may be reduced when DNA is extracted by an outside laboratory.
Meckel-Gruber Syndrome Sequencing Panel (15 genes sequence analysis)

Sample specifications: 3 to 10 cc of blood in a purple top (EDTA) tube
Cost: $2500
CPT codes: 81407
Turn-around time: 8 weeks

Note: We cannot bill insurance for the above test.

Meckel-Gruber Syndrome Deletion/Duplication Panel (13 genes deletion/duplication analysis)

Sample specifications: 3 to 10 cc of blood in a purple top (EDTA) tube
Cost: $1545
CPT codes: 81407
Turn-around time: 6 weeks

Note: The sensitivity of our assay may be reduced when DNA is extracted by an outside laboratory.

Results:
Results, along with an interpretive report, will be faxed to the referring physician. Additional reports will be provided as requested. All abnormal results will be reported by telephone.

For more information about our testing options, please visit our website at dnatesting.uchicago.edu or contact us at 773-834-0555.

References: