



## The University of Chicago Genetic Services Laboratories

5841 S. Maryland Ave., Rm. G701, MC 0077, Chicago, Illinois 60637  
 Toll Free: (888) UC GENES ☐ (888) 824 3637  
 Local: (773) 834 0555 ☐ FAX: (773) 702 9130  
 ucslabs@genetics.uchicago.edu ☐ dnatesting.uchicago.edu  
 CLIA #: 14D0917593 CAP #: 18827-49

### Next Generation Sequencing Panel for Joubert and Meckel Gruber Syndrome

#### 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 interpenduncular 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).

*Our Joubert/Meckel-Gruber Syndrome Panel includes all 26 genes listed below.*

*Our Meckel-Gruber Syndrome Panel includes all 11 genes listed below.*

*(Deletion/duplication analysis is available for most genes; sequencing only is available for starred genes.)*

Joubert/Meckel-Gruber Syndrome				Meckel-Gruber Syndrome	
AHI1	CSPP1*	RPGRIP1L	TMEM237	B9D1*	TMEM67
ARL13B	INPP5E	TCTN1	TTC21B	B9D2*	TMEM216
B9D1*	KIF7	TCTN2		CC2D2A	TMEM231
B9D2*	OFD1	TCTN3		CEP290	
C5orf42	MKS1	TMEM67		MKS1	
CC2D2A	NPHP1	TMEM138		NPHP3*	
CEP41	NPHP3*	TMEM216		RPGRIP1L	
CEP290	PDE6D*	TMEM231		TCTN2	

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 less than 50% of Joubert syndrome cases. Mutations in the Meckel Gruber Syndrome Panel account for approximately 75% of cases

Genes	Associated Disorder(s)	Clinical Features / Molecular Pathology
<i>AHI1</i>	JBTS3	Parisi <i>et al</i> , 2006 identified mutations in <i>AHI1</i> in 13/117 patients with JSRD (3). Valenti <i>et al</i> , 2006, identified <i>AHI1</i> 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 <i>AHI1</i> mutations (5). <i>AHI1</i> 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.
<i>ARL13B</i>	JBTS8	Mutations in <i>ARL13B</i> account for less than 1% of patients with JSRD and the majority of mutations identified in <i>ARL13B</i> to date are missense (6). The <i>ARL13B</i> gene, encoding ADP-ribosylation factor-like protein 13B, is a small GTPase belonging to the class of Arf/Ar1 family within the Ras superfamily of small GTPases involved in diverse cellular functions.

<i>B9D1</i>	MKS9	In a fetus with MKS, Hopp <i>et al</i> (2011) identified a patient who was compound heterozygous for a splice-donor site change in <i>B9D1</i> and a 1.7 Mb <i>de novo</i> deletion encompassing the other allele (7). The fetus inherited an additional likely pathogenic missense change in <i>CEP290</i> from its mother, suggesting possible oligogenic inheritance in this disorder.
<i>B9D2</i>	MKS10	In two fetuses with MKS from a consanguineous family, Dowdle <i>et al</i> (2011) identified a homozygous missense mutation in <i>B9D2</i> (8). Mouse mutations in <i>B9d2</i> compromise ciliogenesis and result in phenotypes consistent with MKS.
<i>C5orf42</i>	JBTS17	Srouf <i>et al</i> , 2012 identified mutations in <i>C5orf42</i> in 7 French Canadian families with JSRD (9). Nonsense, splice site and missense mutations have been identified in <i>C5orf42</i> . The <i>C5orf42</i> protein features a transmembrane protein and is expressed widely in the brain.
<i>CC2D2A</i>	JBTS9, MKS6	Mutations in <i>CC2D2A</i> represent a cause of both JSRD and MKS and are responsible for approximately 10% of each disorder (10). Like <i>AHI1</i> , <i>CC2DA</i> 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.
<i>CEP41</i>	JBTS15	Lee <i>et al</i> , 2012 identified three different homozygous splicing mutations in the <i>CEP41</i> gene in affected members of three consanguineous (Egyptian and Portuguese) families with JSRD (11). Heterozygous missense changes in <i>CEP41</i> have also been reported in several ciliopathies including MKS and Bardet Biedl syndrome.
<i>CEP290</i>	JBTS5, MKS4	Mutations in <i>CEP290</i> account for approximately 20% of patients with JSRD and in a handful of families with MKS and Meckel-like syndrome (12). The vast majority of mutations in <i>CEP290</i> are truncating. Mutations in <i>CEP290</i> have also been identified in individuals with Leber Congenital Amaurosis, Bardet-Biedl syndrome and Senior-Loken syndrome. The <i>CEP290</i> gene encodes a centrosomal protein involved in ciliary assembly and ciliary trafficking.
<i>CSPP1</i>	JBTS21	In 19 patients from 15 families with JSRD, Tuz <i>et al</i> (2014) identified biallelic truncating mutations in <i>CSPP1</i> (13). 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. Zebrafish knockouts of <i>cspp1</i> were consistent with the phenotype observed in other JSRD and resulted in reduced ciliary localization, further supporting the loss of <i>CSPP1</i> as a cause of JSRD.
<i>INPP5E</i>	JBTS	Bielas <i>et al</i> , 2009 identified 5 different homozygous missense mutations in <i>INPP5E</i> in 7 consanguineous families from the United Arab Emirates, Turkey, Egypt and Italy (14). Mutations in <i>INPP5E</i> that cause JSRD are typically missense changes. The <i>INPP5E</i> protein cleaves the 5-position phosphate from soluble inositol phosphates or inositol phospholipids and is localized predominantly to the primary cilium.
<i>KIF7</i>	JBTS12	Truncating mutations in <i>KIF7</i> have been identified in a handful of patients with JBTS (15). The relative contribution of mutations in this gene to JSRD remains unknown, as a limited number of individuals with mutations have been described. The <i>KIF7</i> gene encodes a cilia-associated protein belonging to the kinesin family that plays a role in the hedgehog signaling pathway.
<i>OFD1</i>	JBTS10, OFD1	To date, three families with X-linked Joubert syndrome have been identified with truncating mutations in <i>OFD1</i> (16). Mutations in <i>OFD1</i> 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.
<i>MKS1</i>	MKS1	Mutations in <i>MKS1</i> account for approximately 10% of patients with MKS (2). Truncating, frameshift and splice site mutations have been reported. Missense mutations in <i>MKS1</i> do not appear to be causative for MKS (2). <i>MKS1</i> is widely expressed in the brain, liver, kidney and developing digits.
<i>NPHP1</i>	JBTS4	Parisi <i>et al</i> , identified homozygous and compound heterozygous deletions of the <i>NPHP1</i> gene in individuals with mild JBTS (3, 17). 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 <i>NPHP1</i> are also responsible for 20-30% of the purely renal forms of juvenile nephronophthisis and only about 1-3% of individuals with JSRD (5).
<i>NPHP3</i>	MKS7	In four families in which a total of seven individuals had features consistent with MKS, Bergmann <i>et al</i> (2008) identified homozygous or compound heterozygous mutations in <i>NPHP3</i> (18). Splice-site, nonsense, and missense mutations were observed. Mutations in <i>NPHP3</i> are also responsible for approximately 1% of juvenile nephronophthisis (19).
<i>PDE6D</i>	JBTS22	In a consanguineous family in which three of five siblings were affected with JBTS, Thomas <i>et al</i> (2014) identified a homozygous splice-site mutation leading to an in-frame deletion of <i>PDE6D</i> exon 3 (20). Mutations were not identified in an additional 940 patients with clinically diagnosed ciliopathy conditions, indicating that this is a rare cause of JBTS. <i>Pde6d</i> knockdown in zebrafish was shown to alter both eye and kidney development (20).

<i>RPGRIP1L</i>	JBTS7, MKS5	Mutations in <i>RPGRIP1L</i> account for approximately 2-4% of patients with JSRD and the phenotypic spectrum includes predominantly renal disease (5). Delous <i>et al.</i> , 2007 identified homozygous and compound heterozygous truncating mutations in 3 fetuses with MKS (21). Mutations in <i>RPGRIP1L</i> that cause MKS tend to be protein-truncating and have a more severe effect on protein function.
<i>TCTN1</i>	JBTS13	Garcia-Gonzalo <i>et al.</i> 2011 identified a homozygous splice site mutation in <i>TCTN1</i> in a consanguineous family from Bangladesh (22). The relative contribution of mutations in this gene to JSRD remains unknown, as a limited number of individuals with mutations have been described.
<i>TCTN2</i>	MKS8	A homozygous splice site mutation in <i>TCTN2</i> was identified by Shaheen <i>et al.</i> (2011) in a consanguineous Saudi family with 5 pregnancies affected with MKS (23). <i>Tctn2</i> -null mice lack nodal cilia and have a minimal amount of cilia in the neural tube (22).
<i>TCTN3</i>	JBTS18, OFD4	Thomas <i>et al.</i> (2012) identified a homozygous mutation in <i>TCTN3</i> in a consanguineous Turkish family with two sibilings with Joubert syndrome (24). Homozygous or compound heterozygous mutations in <i>TCTN3</i> have also been associated with oralfaciodigital syndrome type 4 (OFD4) (24). Functional studies of <i>TCTN3</i> have found that it is necessary for transduction of the SHH signaling pathway.
<i>TMEM67</i>	JBTS6, MKS3	Doherty <i>et al.</i> , 2009, identified mutations in <i>TMEM67</i> in 19/232 (8%) of individuals with JSRD (25). Mutations in <i>TMEM67</i> 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).
<i>TMEM138</i>	JBTS16	Lee <i>et al.</i> , 2012 identified missense and splicing mutations in <i>TMEM138</i> in 6 consanguineous Arab families with JSRD (26). 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 <i>TMEM216</i> gene.
<i>TMEM216</i>	JBTS2, MKS2	Valente <i>et al.</i> 2010 identified mutations in <i>TMEM216</i> in 14/462 (14%) of patients with JSRD (27). A common founder mutation (p.R12L) has been identified in the Ashkenazi Jewish population with a carrier rate of 1 in 92 (28). Mutations in <i>TMEM216</i> also account for less than 5% of patients with MKS (2).
<i>TMEM231</i>	JBTS20	Srouf <i>et al.</i> (2012) identified compound heterozygous mutations in <i>TMEM231</i> in 3 individuals with Joubert syndrome from two unrelated French Canadian families (29). Knockout <i>Tmem231</i> mice show embryonic lethality with microphthalmia, polydactyly and patterning defects of the ventral spinal cord, consistent with a ciliopathy (30).
<i>TMEM237</i>	JBST14	Huang <i>et al.</i> , 2011 identified homozygous truncating mutations in the <i>TMEM237</i> in 10 related Canadian Hutterite families with JSRD (31). Screening of an additional 201 individuals with JSRD and 90 individuals with MKS identified two additional patients with mutations in <i>TMEM237</i> .
<i>TTC21B</i>	JATD, NPHP JSRD	Davis <i>et al.</i> 2011 sequenced <i>TTC21B</i> 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 (32). 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.

### 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 (26 genes, sequence analysis)

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	\$3890
CPT codes:	81407
Turn-around time:	8 weeks

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

### Joubert/Meckel-Gruber Deletion/Duplication Panel (21 genes (see above), 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 (11 genes, sequence analysis)

Sample specifications:	3 to 10 cc of blood in a purple top (EDTA) tube
Cost:	\$2890
CPT codes:	81407
Turn-around time:	8 weeks

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

### Meckel-Gruber Syndrome Deletion/Duplication Panel (8 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.

### **References:**

1. Parisi M, Glass I. Joubert Syndrome and Related Disorders. In: Pagon R, Bird T, Dolan C, eds. GeneReviews [Internet]. Seattle: University of Washington, 2003.
2. Logan CV, Abdel-Hamed Z, Johnson CA. Molecular genetics and pathogenic mechanisms for the severe ciliopathies: insights into neurodevelopment and pathogenesis of neural tube defects. *Mol Neurobiol* 2011; 43: 12-26.
3. Parisi MA, Doherty D, Eckert ML et al. AHI1 mutations cause both retinal dystrophy and renal cystic disease in Joubert syndrome. *J Med Genet* 2006; 43: 334-339.
4. Valente EM, Brancati F, Silhavy JL et al. AHI1 gene mutations cause specific forms of Joubert syndrome-related disorders. *Ann Neurol* 2006; 59: 527-534.
5. Parisi MA. Clinical and molecular features of Joubert syndrome and related disorders. *Am J Med Genet C Semin Med Genet* 2009; 151C: 326-340.
6. Cantagrel V, Silhavy JL, Bielas SL et al. Mutations in the cilia gene ARL13B lead to the classical form of Joubert syndrome. *Am J Hum Genet* 2008; 83: 170-179.
7. Hopp K, Heyer CM, Hommerding CJ et al. B9D1 is revealed as a novel Meckel syndrome (MKS) gene by targeted exon-enriched next-generation sequencing and deletion analysis. 2011.
8. Dowdle WE, Robinson JF, Kneist A et al. Disruption of a ciliary B9 protein complex causes Meckel syndrome. *Am J Hum Genet* 2011; 89: 94-110.
9. Srour M, Schwartzentruber J, Hamdan FF et al. Mutations in C5ORF42 cause Joubert syndrome in the French Canadian population. *Am J Hum Genet* 2012; 90: 693-700.
10. Bachmann-Gagescu R, Ishak GE, Dempsey JC et al. Genotype-phenotype correlation in CC2D2A-related Joubert syndrome reveals an association with ventriculomegaly and seizures. *J Med Genet* 2012; 49: 126-137.
11. Lee JE, Silhavy JL, Zaki MS et al. CEP41 is mutated in Joubert syndrome and is required for tubulin glutamylation at the cilium. *Nat Genet* 2012; 44: 193-199.
12. Coppeters F, Lefever S, Leroy BP et al. CEP290, a gene with many faces: mutation overview and presentation of CEP290base. *Hum Mutat* 2010; 31: 1097-1108.
13. Tuz K, Bachmann-Gagescu R, O'Day DR et al. Mutations in CSPP1 cause primary cilia abnormalities and Joubert syndrome with or without Jeune asphyxiating thoracic dystrophy. *Am J Hum Genet* 2014; 94: 62-72.
14. Bielas SL, Silhavy JL, Brancati F et al. Mutations in INPP5E, encoding inositol polyphosphate-5-phosphatase E, link phosphatidylinositol signaling to the ciliopathies. *Nat Genet* 2009; 41: 1032-1036.
15. Dafinger C, Liebau MC, Elsayed SM et al. Mutations in KIF7 link Joubert syndrome with Sonic Hedgehog signaling and microtubule dynamics. *J Clin Invest* 2011; 121: 2662-2667.
16. Field M, Scheffer IE, Gill D et al. Expanding the molecular basis and phenotypic spectrum of X-linked Joubert syndrome associated with OFD1 mutations. *Eur J Hum Genet* 2012; 20: 806-809.
17. Parisi MA, Bennett CL, Eckert ML et al. The NPHP1 gene deletion associated with juvenile nephronophthisis is present in a subset of individuals with Joubert syndrome. *Am J Hum Genet* 2004; 75: 82-91.
18. Bergmann C, Fliegauf M, Bruchle NO et al. Loss of nephrocystin-3 function can cause embryonic lethality, Meckel-Gruber-like syndrome, situs inversus, and renal-hepatic-pancreatic dysplasia. *Am J Hum Genet* 2008; 82: 959-970.
19. Hildebrandt F, Attanasio M, Otto E. Nephronophthisis: disease mechanisms of a ciliopathy. *J Am Soc Nephrol* 2009; 20: 23-35.
20. Thomas S, Wright KJ, Le Corre S et al. A homozygous PDE6D mutation in Joubert syndrome impairs targeting of farnesylated INPP5E protein to the primary cilium. *Hum Mutat* 2014; 35: 137-146.
21. Delous M, Baala L, Salomon R et al. The ciliary gene RPGRIP1L is mutated in cerebello-oculo-renal syndrome (Joubert syndrome type B) and Meckel syndrome. *Nat Genet* 2007; 39: 875-881.
22. Garcia-Gonzalo FR, Corbit KC, Sirerol-Piquer MS et al. A transition zone complex regulates mammalian ciliogenesis and ciliary membrane composition. *Nat Genet* 2011; 43: 776-784.
23. Shaheen R, Faqeih E, Seidahmed MZ et al. A TCTN2 mutation defines a novel Meckel Gruber syndrome locus. *Hum Mutat* 2011; 32: 573-578.
24. Thomas S, Legendre M, Saunier S et al. TCTN3 mutations cause Mohr-Majewski syndrome. *Am J Hum Genet* 2012; 91: 372-378.
25. Doherty D, Parisi MA, Finn LS et al. Mutations in 3 genes (MKS3, CC2D2A and RPGRIP1L) cause COACH syndrome (Joubert syndrome with congenital hepatic fibrosis). *J Med Genet* 2010; 47: 8-21.
26. Lee JH, Silhavy JL, Lee JE et al. Evolutionarily assembled cis-regulatory module at a human ciliopathy locus. *Science* 2012; 335: 966-969.
27. Valente EM, Logan CV, Mougou-Zerelli S et al. Mutations in TMEM216 perturb ciliogenesis and cause Joubert, Meckel and related syndromes. *Nat Genet* 2010; 42: 619-625.
28. Edvardson S, Shaag A, Zenvirt S et al. Joubert syndrome 2 (JBTS2) in Ashkenazi Jews is associated with a TMEM216 mutation. *Am J Hum Genet* 2010; 86: 93-97.
29. Srour M, Hamdan FF, Schwartzentruber JA et al. Mutations in TMEM231 cause Joubert syndrome in French Canadians. *J Med Genet* 2012; 49: 636-641.
30. Chih B, Liu P, Chinn Y et al. A ciliopathy complex at the transition zone protects the cilia as a privileged membrane domain. *Nat Cell Biol* 2012; 14: 61-72.
31. Huang L, Szymanska K, Jensen VL et al. TMEM237 is mutated in individuals with a Joubert syndrome related disorder and expands the role of the TMEM family at the ciliary transition zone. *Am J Hum Genet* 2011; 89: 713-730.
32. Davis EE, Zhang Q, Liu Q et al. TTC21B contributes both causal and modifying alleles across the ciliopathy spectrum. *Nat Genet* 2011; 43: 189-196.