



**Molecular Testing for Lissencephaly**

**Clinical Features:**

Classic Lissencephaly (LIS) or Lissencephaly Type 1 is a smooth or nearly smooth cerebral surface caused by deficient neuronal migration. The spectrum of malformations ranges from complete agyria (absent gyri) to regional pachygyria to subcortical band heterotopia (SBH).

- Lissencephaly—“smooth brain” with absent (agyria) or abnormally wide gyri (pachygyria)
- SBH—“double cortex”; band of heterotopic gray matter below the cortex separated by a thin zone of normal white matter
- Miller-Dieker syndrome—lissencephaly, characteristic facial features and severe neurologic abnormalities
- X-linked lissencephaly with abnormal genitalia (XLAG)—lissencephaly and moderately increased thickness of the cortex, absence of the corpus callosum, infantile spasms, hypothalamic dysfunction including deficient temperature regulation, and ambiguous genitalia in males.

Lissencephaly and SBH are classified by anterior-posterior gradient and severity. This classification may help determine the best order for genetic testing.

*Dr. William Dobyns at the Seattle Children’s Research Institute is available to review MRI scans and give recommendations regarding genetic testing. Please contact Dr. Dobyns ([wbd@uw.edu](mailto:wbd@uw.edu)) to arrange this, if desired.*

*Our Lissencephaly Sequencing Panel includes sequencing of all 37 genes listed below. Our Comprehensive Lissencephaly Panel includes sequencing of all 37 genes listed below and deletion/duplication analysis of the genes listed below in **bold**.*

| Lissencephaly Panel |                |                 |               |               |
|---------------------|----------------|-----------------|---------------|---------------|
| <b>ACTB</b>         | <b>DYNC1H1</b> | <b>LAMA2</b>    | <b>POMT1</b>  | <b>TUBB</b>   |
| <b>ACTG1</b>        | <b>FKRP</b>    | <b>LAMB1</b>    | <b>POMT2</b>  | <b>TUBB2B</b> |
| <b>ARX</b>          | <b>FKTN</b>    | <b>LARGE</b>    | <b>RELN</b>   | <b>TUBB3</b>  |
| <b>ATP6V0A2</b>     | <b>GMPPB</b>   | <b>NDE1</b>     | <b>SGK196</b> | <b>TUBG1</b>  |
| <b>B3GALNT2</b>     | <b>GPR56</b>   | <b>PAFAH1B1</b> | <b>SNAP29</b> | <b>VLDLR</b>  |
| <b>B3GNT1</b>       | <b>ISPD</b>    | <b>POMGNT1</b>  | <b>SRD5A3</b> |               |
| <b>CDK5</b>         | <b>KATNB1</b>  | <b>POMGNT2</b>  | <b>TMEM5</b>  |               |
| <b>DCX</b>          | <b>KIF5C</b>   | <b>POMK</b>     | <b>TUBA1A</b> |               |

*Our Cobblestone Lissencephaly Sequencing Panel includes sequencing of all 19 genes listed below.*

| Cobblestone Lissencephaly Sequencing Panel |              |                |               |
|--|--------------|----------------|---------------|
| <b>ATP6V0A2</b>                            | <b>GMPPB</b> | <b>LARGE</b>   | <b>POMT2</b>  |
| <b>B3GALNT2</b>                            | <b>GPR56</b> | <b>POMGNT1</b> | <b>SNAP29</b> |
| <b>B3GNT1</b>                              | <b>ISPD</b>  | <b>POMGNT2</b> | <b>SRD5A3</b> |
| <b>FKRP</b>                                | <b>LAMA2</b> | <b>POMK</b>    | <b>TMEM5</b>  |
| <b>FKTN</b>                                | <b>LAMB1</b> | <b>POMT1</b>   |               |

**Molecular Genetics:**

The genetic causes of lissencephaly are complex, and may result from abnormalities in one of the genes below:

| Gene / Condition            | Inheritance Pattern | Clinical Features and Molecular Pathology   |
|-----------------------------|---------------------|---|
| <b>ACTB</b><br><b>ACTG1</b> | AD                  | Baraitser-Winter syndrome is a developmental disorder characterized by congenital ptosis, high-arched eyebrows, hypertelorism, ocular colobomata and anterior-predominant lissencephaly. Other features include postnatal short stature, microcephaly, ID, seizures and hearing loss (1). Mutations in both <b>ACTB</b> and <b>ACTG1</b> , which code for cytoplasmic actin, have been identified in patients with Baraitser-Winter syndrome. |

|   |          |   |
|---|----------|---|
| ARX   | X-linked | ARX mutations cause various phenotypes including XLAG, X-linked infantile spasms, and non-syndromic X-linked mental retardation (2-4). Females with more severe mutations may be affected as well, and have agenesis of the corpus callosum and seizures (5).   |
| ATP6V0A2  | AR       | Homozygous or compound heterozygous mutations in the <i>ATP6V0A2</i> gene cause autosomal recessive cutis laxa type IIA (ARCL2A). This condition is characterized by overfolding and wrinkling of the skin and dysmorphic craniofacial features. Individuals with ARCL2A have early developmental delays, and seizures associated with a neurodegenerative course (6). Van Maldergem <i>et al.</i> (2008) reported cortical malformations reminiscent of Walker-Warburg syndrome in 8 patients with ARCL2A (6).   |
| B3GALNT2<br>B3GNT1<br>FKTN<br>FKRP<br>GMPPB<br>LARGE<br>POMGNT1<br>POMGNT2<br>POMK<br>POMT1<br>POMT2<br>ISPD<br>TMEM5 | AR       | Congenital muscular dystrophy-dystroglycanopathy with brain and eye anomalies type A (MDDGA) is a genetically heterogeneous group of autosomal recessive conditions caused by defective glycosylation of DAG1, including Walker-Warburg syndrome, muscle-eye-brain and Fukuyama muscular dystrophy. Features of these conditions include brain and eye malformations, cognitive impairment, and congenital muscular dystrophy. Brain malformations seen in MDDGA include cobblestone lissencephaly, polymicrogyria, hydrocephalus, and cerebellar hypoplasia. Cobblestone lissencephaly (COB, previously designated as lissencephaly "type 2"), is a brain malformation consisting of a complex cortical dysplasia with glioneuronal heterotopia on the brain surface, moderate to severe lissencephaly, dysmyelination, hypoplastic brainstem, and dysplastic cerebellum with cysts. Mutations in <i>FKTN</i> , <i>FKRP</i> , <i>LARGE</i> , <i>POMGnT1</i> , <i>POMT1</i> and <i>POMT2</i> account for approximately 32-50% of patients with cobblestone lissencephaly (7). Mutations in <i>TMEM5</i> and <i>ISPD</i> account for approximately 20% of patients with cobblestone lissencephaly (8). |
| CDK5  | AR       | In a consanguineous family with individuals affected by severe lissencephaly, cerebellar hypoplasia and agenesis of the corpus callosum, Magen <i>et al.</i> (2015) identified a homozygous truncating variant in the <i>CDK5</i> gene (9).   |
| DCX   | X-linked | <i>DCX</i> abnormalities result in severe lissencephaly or SBH in boys, but a less severe SBH in girls (10). <i>DCX</i> abnormalities are generally associated with an a>p gradient. In males, <i>DCX</i> mutations are present in approximately 30% with SBH and approximately 10% with lissencephaly. In females, <i>DCX</i> mutations are present in approximately 80% with SBH, especially those with diffuse bands or bilateral frontal only bands. Intragenic deletions of the <i>DCX</i> gene are present in approximately 10% of female patients with SBH in whom no mutations were identified by <i>DCX</i> sequencing (11, 12).   |
| DYNC1H1   | AD       | Heterozygous mutations in <i>DYNC1H1</i> are associated with cortical malformations including pachygyria, polymicrogyria and nodular heterotopia (13).  |
| GPR56   | AR       | Biallelic mutations in <i>GPR56</i> have been associated with a range of phenotypes, from bilateral bifrontoparietal polymicrogyria, to cobblestone-like lissencephaly (14).  |
| KATNB1  | AR       | Homozygous mutations in <i>KATNB1</i> have been identified in multiple unrelated consanguineous families with variable malformations in cortical development, including lissencephaly, pachygyria, and hypoplasia of the corpus callosum (15).  |
| KIF5C   | AD       | Heterozygous mutations in <i>KIF5C</i> have been associated with severe cortical malformations including gyral simplification and polymicrogyria (16).  |
| LAMA2   | AR       | <i>LAMA2</i> -related muscular dystrophy is an autosomal recessive group of conditions ranging from late-onset proximal weakness and motor delays to profound neonatal hypotonia, failure to thrive, ophthalmoparesis, and respiratory failure. A small proportion of individuals with early-onset <i>LAMA2</i> -related muscular dystrophy have brain malformations, including pachygyria and cortical dysplasia (18). The majority of affected individuals have normal cognitive abilities and cognitive development is not consistently correlated with brain MRI findings (19).   |
| LAMB1   | AR       | In two consanguineous families with cobblestone lissencephaly, Radmanesh <i>et al.</i> (2013) identified two different homozygous loss-of-function mutations in the <i>LAMB1</i> gene (20). Radmanesh <i>et al.</i> noted that although the brain malformations were similar to those identified in patients with muscular-dystrophy-dystroglycanopathies, these patients did not have significant eye or muscle disease.   |
| NDE1  | AR       | Mutations in <i>NDE1</i> have been reported in children with severe congenital microcephaly, with brains smaller than 10 SD below the mean, with simplified gyri, and profound developmental handicap with normal body growth. Patients may also have lissencephaly or microhydraencephaly. Paciorkowski, <i>et al.</i> (2013) reported a patient with a full gene deletion and a truncating mutation in <i>NDE1</i> who had severe microcephaly, agenesis of the corpus callosum, and a cortical dysplasia with a polymicrogyria-like appearance (21). <i>NDE1</i> is highly expressed in the developing human and mouse cerebral cortex, particularly at the centrosome, and has a role in  |

|                             |    |   |
|-----------------------------|----|---|
|                             |    | mitotic spindle assembly during early neurogenesis. Deficiency of NDE1 therefore appears to cause failure of neurogenesis and a deficiency of cortical lamination.  |
| <i>PAFAH1B1 (LIS1)</i>      | AD | <i>PAFAH1B1 (LIS1)</i> abnormalities cause the most severe form of lissencephaly and are generally associated with a p>a gradient (22). <i>PAFAH1B1</i> mutations are present in approximately 30% of patients with <i>PAFAH1B1</i> -related lissencephaly and rarely in patients with SBH. Microdeletions involving 17p13.3 are present in 100% of patients with MDS and approximately 50% of patients with lissencephaly. Intragenic deletions of one or more exons of <i>LIS1</i> are present in approximately 15% of patients with <i>PAFAH1B1</i> -related lissencephaly (23).   |
| <i>RELN</i><br><i>VLDLR</i> | AR | <i>RELN</i> mutations have been identified in patients with a less severe form of lissencephaly with cerebellar hypoplasia (LCH) (24). <i>VLDLR</i> -associated cerebellar hypoplasia ( <i>VLDLR</i> -CH) falls within the LCH spectrum, and is characterized by non-progressive congenital ataxia, ID, dysarthria, strabismus and seizures. These patients have mild lissencephaly as well. <i>VLDR</i> is part of the reelin ( <i>RELN</i> ) signaling pathway, which guides neuroblast migration in the cerebral cortex and cerebellum. LCH is distinguished from <i>VLDLR</i> -CH by more severe lissencephaly with an a>p gradient, a small and malformed hippocampus, and profound cerebellar hypoplasia with complete absence of detectable folia (25).  |
| <i>SNAP29</i>               | AR | Sprecher <i>et al</i> (2005) identified a homozygous truncating mutation in two unrelated consanguineous Arab Muslim families with cerebral dysgenesis, neuropathy, ichthyosis, and palmoplantar keratoderma (CEDNIK) syndrome. Individuals with CEDNIK syndrome have progressive microcephaly in conjunction with a range of brain malformations, including cortical dysplasia, pachygyria, absence of the corpus callosum, and perisylvian polymicrogyria. Additional features include dysmorphic facies, palmoplantar keratosis and ichthyosis, severe developmental delays, optic disc hypoplasia, and sensorineural hearing loss (26).   |
| <i>SRD5A3</i>               | AR | Mutations in <i>SRD5A3</i> are associated with congenital disorder of glycosylation type Iq (CDG1Q). CDG1Q is a rare autosomal recessive condition characterized by abnormal type 1 glycosylation in association with congenital eye malformations including ocular colobomas and optic disc hypoplasia, intellectual disabilities, and variable brain malformations. Al-Gazali <i>et al.</i> (2008) reported a consanguineous family with multiple affected individuals. Brain malformations seen in affected individuals included cerebellar vermis hypoplasia, hypoplasia of the corpus callosum, absent septum pellucidum, and bilateral frontal polymicrogyria (27). Cantagrel <i>et al.</i> (2010) subsequently identified a homozygous mutation in <i>SRD5A3</i> in affected individuals in this family, as well as homozygous or compound heterozygous <i>SRD5A3</i> mutations in 5 other individuals (28). |
| <i>TUBA1A</i>               | AD | <i>TUBA1A</i> mutations have been identified in patients with gyral malformations and are associated with two forms of lissencephaly. The first is lissencephaly with a p>a gradient similar to <i>LIS1</i> -associated lissencephaly, although this is rare cause of typical lissencephaly. The second is a severe form of lissencephaly associated with severe cerebellar hypoplasia (LCH) and often underdevelopment of the corpus callosum. About 30-40% of children with LCH have mutations in <i>TUBA1A</i> . (29).   |
| <i>TUBB</i><br><i>TUBB3</i> | AD | Pathogenic variants in <i>TUBB</i> (previously known as <i>TUBB5</i> ) and <i>TUBB3</i> have been associated with cortical malformations including simplified gyral patterns (30).  |
| <i>TUBB2B</i>               | AD | <i>De novo</i> mutations in the <i>TUBB2B</i> gene have been associated with a range of cortical malformations, including polymicrogyria and lissencephaly (31).  |
| <i>TUBG1</i>                | AD | Heterozygous mutations in <i>TUBG1</i> have been associated with moderate to severe lissencephaly and subcortical band heterotopia (30).  |

### Test methods:

Comprehensive sequence coverage of the coding regions and splice junctions of all genes in this panel is performed. Targets of interests 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 pathogenic and likely 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 MLPA or 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.

For patients of Asian descent that order the Lissencephaly or Cobblestone Lissencephaly panels: Two common non-coding mutations in the *FKTN* gene, present in individuals of Asian descent, including the retrotransposon

insertion in the 3'UTR region and the c.647+2084G>T mutation in intron 5 will also be analyzed. For the former, PCR amplification is performed to produce a unique, specific, 400 bp amplification product specific for the retrotransposon insertion. For the latter, a portion of intron 5 is PCR amplified and sequenced in both the forward and reverse directions and analyzed for the c.647+2084G>T mutation.

Comprehensive Lissencephaly Panel (37 genes sequencing, 33 genes deletion/duplication analysis)

|                        |  |
|------------------------|--|
| Sample specifications: | 3 to10 cc of blood in a purple top (EDTA) tube |
| Cost:                  | \$4000   |
| CPT codes:             | 81406, 81407                                   |
| Turn-around time:      | 6 weeks  |

Lissencephaly Sequencing Panel (37 genes sequence analysis)

|                        |  |
|------------------------|--|
| Sample specifications: | 3 to10 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.**

Lissencephaly Deletion/Duplication Panel (33 genes deletion/duplication analysis)

|                        |  |
|------------------------|--|
| Sample specifications: | 3 to10 cc of blood in a purple top (EDTA) tube |
| Cost:                  | \$2500   |
| CPT codes:             | 81407  |
| Turn-around time:      | 6 weeks  |

Cobblestone Lissencephaly Sequencing panel (19 sequencing analysis)

|                        |  |
|------------------------|--|
| Sample specifications: | 3 to10 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.**

Cobblestone Lissencephaly Del/Dup panel (19 genes deletion/duplication analysis)

|                        |  |
|------------------------|--|
| Sample specifications: | 3 to10 cc of blood in a purple top (EDTA) tube |
| Cost:                  | \$1545   |
| CPT codes:             | 81407  |
| Turn-around time:      | 6 weeks  |

## 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](http://dnatesting.uchicago.edu) or contact us at 773-834-0555.***

## References

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