Lissencephaly Panel
Sequence Analysis and Exon-Level Deletion/Duplication Testing of 26 Genes

Panel Gene List

ACTB, ACTG1, ARX*, ATP6V0A2, B3GALNT2, B4GAT1**, CIT, DCX, FKRP**, FKTN, GMPPB, ISPD, KATNB1, LAMB1, LARGE1, NDE1, PAFAH1B1, POMGNT1*, POMGNT2, POMT1, POMT2, RELN, TMEM5, TUBA1A*, VLDLR, WDR62*

*Only large deletion/duplications may detected for the *ACTB, ARX, POMGNT1* and *TUBA1A* genes

**No deletion/duplication analysis for the *B4GAT1* and *FKRP* genes

Clinical Features

Lissencephaly is characterized by a thickened cortex and the absence of folds or gyri (agyria) or the presence of abnormally wide gyri (pachygyria). Lissencephalies can present in different forms and with varying severity, ranging from complete agyria to mixed agyria and pachygyria, or simplified gyri with subcortical band heterotopia (double cortex). Pathogenic variants in distinct genes lead to overlapping forms of lissencephaly, including classic smooth lissencephaly, cobblestone lissencephaly, lissencephaly with agenesis of the corpus callosum, and lissencephaly with cerebellar hypoplasia. Subcortical band heterotopia is a mild form of lissencephaly characterized by normal gyri but the presence of an abnormal and often symmetric band of gray matter under the cortex. Lissencephalies uniformly cause developmental delay, epilepsy, and intellectual disability.[1] The spectrum of disorders associated with lissencephaly includes a group of congenital muscular dystrophies called the alpha-dystroglycanopathies including Walker-Warburg syndrome which is characterized by cobblestone lissencephaly, cerebellar abnormalities, eye defects, muscle weakness.[2,3]

Genetics

The various forms of lissencephaly demonstrate clinical and genetic heterogeneity.[1,2] Classic lissencephaly occurs as an autosomal dominant, autosomal recessive, or X-linked trait. *PAFAH1B1* (*LIS1*) is among the most prominent genes associated with lissencephaly. Loss of *PAFAH1B1* alone causes epilepsy and developmental delay, but with absence of severe dysmorphism. Deletion of this gene along with adjacent ones in the terminal end of the short arm of chromosome 17 causes Miller-Dieker syndrome, characterized by epilepsy, dysmorphic features, severe developmental delay and, occasionally, heart defects and omphalocele. Somatic mutations in PAFAH1B1 and DCX have been described.[4,5] Cobblestone lissencephaly and lissencephaly with cerebellar hypoplasia segregate as autosomal recessive disorders and lissencephaly with agenesis of the corpus callosum is an X-linked disorder. Lissencephaly can sometimes occur together with heterotopias or polymicrogyria, depending on the affected gene and the type of pathogenic variant.

Test Methods

Using genomic DNA from the submitted specimen, the complete coding regions and splice site junctions of the genes on this panel are enriched using a proprietary targeted capture system developed by GeneDx for next-generation sequencing with CNV calling (NGS-CNV). The enriched targets are simultaneously sequenced with paired-end reads on an Illumina platform. Bi-directional sequence reads are assembled and aligned to reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19. After gene specific filtering, data are analyzed to identify sequence variants and most deletions and duplications involving coding exons; however, technical limitations and inherent sequence properties effectively reduce this resolution for some genes. Alternative sequencing or copy number detection methods are used to analyze or confirm regions with inadequate sequence or copy number data by NGS. Reportable variants include pathogenic variants, likely pathogenic variants and variants of uncertain significance. Likely benign and benign variants, if present, are not routinely reported but are available upon request.

Test Sensitivity

The clinical sensitivity of sequencing and deletion/duplication analysis of the genes included in this panel depends in part on the patient's clinical phenotype. Specific information about the diagnostic yield for each gene in selected populations is summarized in the following table(s). The technical sensitivity of sequencing is estimated to be > 99% at detecting single nucleotide events. It will not reliably detect deletions greater than 20 base pairs, insertions or rearrangements greater than 10 base pairs, or low-level mosaicism. The copy number assessment methods used with this test cannot reliably detect copy number variants of less than 500 base pairs or mosaicism and cannot identify balanced chromosome aberrations. Assessment of exon-level copy number events is dependent on the inherent sequence properties of the targeted regions, including shared homology and exon size. Gene specific exclusions for exon-level deletion/duplication testing for this panel are: *B4GAT1* and *FKRP* genes, no copy number testing, *ACTB, ARX, POMGNT1* and *TUBA1A* genes, only whole gene deletions or duplications may be detected.

Gene	Protein	Inheritance	Disease Associations
*ACTB**	Actin, Beta	AD	80% of Baraitser-Winter syndrome[6]
ACTG1	Actin, Gamma-1	AD	20% of Baraitser-Winter syndrome[6]
*ARX**	Aristaless-related homeobox protein	XL	70-95% of XLAG7,[8],3-10% in XLID[9,10]
ATP6V0A2	Lysosomal H(+)-ATPase V0 subunit A2	AR	21-24% of autosomal recessive cutis laxatype II[11,12]
B3GALNT2	Beta-1,3-N-Acetylgalactosaminyltransferase 2	AR	Rare in alpha-dystroglycanopathies[13] Rare in congenital muscular dystrophy[14]
*B4GAT1 (B3GNT1)***	Beta-1,4-Glucuronyltransferase 1	AR	Rare in alpha-dystroglycanopathies[15,16]
CIT	Citron rho-interacting serine/threonine kinase	AR	Rare in MCPH17,[18,19]
DCX	Doublecortin	XL	Up to 100% XL lissencephaly 10% of classic lissencephaly 85% females and ~30% males with SBH [1,8,20]
*FKRP***	Fukutin-related protein	AR	~2% of cobblestone lissencephaly[3,21] 9% of alpha-dystroglycanopathies[22] 6% of limb-girdle MD[23] 2.5% of congenital muscular dystrophy[14]
FKTN	Fukutin	AR	~7% of alpha-dystroglycanopathies[24] Rare in congenital muscular dystrophy[14] 1.5% of limb-girdle MD[25] Does not include the Japanese founder mutation in the 3' UTR[26]

GMPPB	GDP-Mannose Pyrophosphorylase B	AR	Rare in alpha-dystroglycanopathies[27,28] Rare in congenital muscular dystrophy[14]
ISPD	Isoprenoid synthase domain-containing protein	AR	~6% of cobblestone lissencephaly[24] ~30% of Walker-Warburg syndrome and ~11% of alpha-dystroglycanopathies[29,30] Rare in LGMD31
KATNB1	Katanin regulatory subunit B1	AR	<1% of MCD[32,33]
LAMB1	Laminin, Beta-1	AR	Rare in cobblestone lissencephaly[34]
LARGE1	Like-glycosyltransferase	AR	2-5% of cobblestonelissencephaly[3,21] ~1% of alpha-dystroglycanopathies[22,24]
NDE1	nudE neurodevelopment protein 1	AR	Rare[35,36,37]
PAFAH1B1 (LIS1)	Platelet-Activating Factor Acetylhydrolase 1b, Regulatory Subunit 1	AD	~40-65% of classic lissencephaly[1,38]
*POMGNT1**	Protein O-Mannose Beta 1-2-N-Acetylglucosaminyltransferase	AR	11-18% ofcobblestonelissencephaly[3,21] 8-10% of alpha-dystroglycanopathies[22,24]
POMGNT2	Protein O-Mannose Beta-1,4-N-Acetylglucosaminyltransferase 2	AR	Rare in alpha-dystroglycanopathies[39]
POMT1	Protein O-Mannosyltransferase 1	AR	27-34% ofcobblestonelissencephaly[3,21] 9-21% of alpha-dystroglycanopathies[22,24]
POMT2	Protein O-Mannosyltransferase 2	AR	8-11% of cobblestonelissencephaly[3,21] 9-11% of alpha-dystroglycanopath [22,24]
RELN	Reelin	AR	Rare [40,41]
TMEM5	Transmembrane protein 5	AR	~6% of cobblestone lissencephaly [21] Rare in alpha-dystroglycanopathies[42]
*TUBA1A**	Tubulin, Alpha-1A	AD	1% of classic lissencephaly 30% of lissencephaly with cerebellar hypoplasia [43,44]
VLDLR	Very low density lipoprotein receptor	AR	Rare cerebellar hypoplasia with simplified gyri [45,46]
WDR62	WD repeat-containing protein 62	AR	Unknown[47]

*Only large deletion/duplications may detected for the *ACTB, ARX, POMGNT1* and *TUBA1A* genes
**No deletion/duplication analysis for the *B4GAT1* and *FKRP* genes

REFERENCES:

1. Fry et al. (2014) American Journal Of Medical Genetics. Part C, Seminars In Medical Genetics 166C (2):198-210 (PMID: 24862549).
2.Dyment et al. (2013) Current Neurology And Neuroscience Reports 13 (8):364 (PMID: 23793931)
3.Devisme et al. (2012) Brain : A Journal Of Neurology 135 (Pt 2):469-82 (PMID: 22323514)
4.Dobyns WB, Das S. LIS1-Associated Lissencephaly/Subcortical Band Heterotopia. 2009 Mar 3 [Updated 2014 Aug 14]. In: Pagon RA, Adam MP, Ardinger HH, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2015.Available from: http://www.ncbi.nlm.nih.gov/books/NBK5189/
5.Hehr U, Uyanik G, Aigner L, et al. DCX-Related Disorders. 2007 Oct 19 [Updated 2011 Mar 24]. In: Pagon RA, Adam MP, ArdingerHH, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2015. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1185/
6.Verloes et al. (2015) European Journal Of Human Genetics : Ejhg 23 (3):292-301 (PMID: 25052316)
7.Kato et al. (2004) Human Mutation 23 (2):147-59 (PMID: 14722918)
8.Dobyns (2010) Epilepsia 51 Suppl 1 :5-9 (PMID: 20331703)
9.Poirier et al. (2006) Neurogenetics 7 (1):39-46 (PMID: 16235064)
10.Nawara et al. (2006) American Journal Of Medical Genetics. Part A 140 (7):727-32 (PMID: 16523516)
11.Gardeitchik et al. (2014) European Journal Of Human Genetics : Ejhg 22 (7):888-95 (PMID: 23963297)
12.Fischer et al. (2012) Human Genetics 131 (11):1761-73 (PMID: 22773132)
13.Stevens et al. (2013) American Journal Of Human Genetics 92 (3):354-65 (PMID: 23453667)
14.Sframeli et al. (2017) Neuromuscul. Disord. 27 (9):793-803 (PMID: 28688748)
15.Buysse et al. (2013) Human Molecular Genetics 22 (9):1746-54 (PMID: 23359570)
16.Shaheen et al. (2013) Neurogenetics 14 (3-4):243-5 (PMID: 23877401)
17.Li et al. (2016) Am. J. Hum. Genet. 99 (2):501-10 (PMID: 27453578)
18.Basit et al. (2016) Hum. Genet. 135 (10):1199-207 (PMID: 27519304)
19.Harding et al. (2016) Am. J. Hum. Genet. 99 (2):511-20 (PMID: 27453579)
20.Bahi-Buisson et al. (2013) Brain : A Journal Of Neurology 136 (Pt 1):223-44 (PMID: 23365099)
21.Vuillaumier-Barrot et al. (2012) American Journal Of Human Genetics 91 (6):1135-43 (PMID: 23217329)
22.Mercuri et al. (2009) Neurology 72 (21):1802-9 (PMID: 19299310)
23.Pegoraro E, Hoffman EP. Limb-Girdle Muscular Dystrophy Overview. 2000 Jun 8 [Updated 2012 Aug 30]. In: Pagon RA, Adam MP,Ardinger HH, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2015. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1408/
24.Godfrey et al. (2007) Brain : A Journal Of Neurology 130 (Pt 10):2725-35 (PMID: 17878207)
25.Oestergaard et al. (2016) Neurol Genet 2 (6):e112 (PMID: 27766311)
26.Saito K. Fukuyama Congenital Muscular Dystrophy. 2006 Jan 26 [Updated 2012 May 10]. In: Pagon RA, Adam MP, Ardinger HH, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2015. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1206/
27.Jensen et al. (2015) Hum. Mutat. : (PMID: 26310427)
28.Carss et al. (2013) American Journal Of Human Genetics 93 (1):29-41 (PMID: 23768512)
29.Czeschik et al. (2013) European Journal Of Medical Genetics 56 (12):689-94 (PMID: 24120487)
30.Willer et al. (2012) Nature Genetics 44 (5):575-80 (PMID: 22522420)
31.Cirak et al. (2013) Brain : A Journal Of Neurology 136 (Pt 1):269-81 (PMID: 23288328)
32.Mishra-Gorur et al. (2014) Neuron 84 (6):1226-39 (PMID: 25521378)
33.Hu et al. (2014) Neuron 84 (6):1240-57 (PMID: 25521379)
34.Radmanesh et al. (2013) American Journal Of Human Genetics 92 (3):468-74 (PMID: 23472759)
35.Bakircioglu et al. (2011) American Journal Of Human Genetics 88 (5):523-35 (PMID: 21529752)
36.Alkuraya et al. (2011) American Journal Of Human Genetics 88 (5):536-47 (PMID: 21529751)
37.Guven et al. (2012) Neurogenetics 13 (3):189-94 (PMID: 22526350)
38.Saillour et al. (2009) Archives Of Neurology 66 (8):1007-15 (PMID: 19667223)
39.Manzini et al. (2012) American Journal Of Human Genetics 91 (3):541-7 (PMID: 22958903)
40.Hong et al. (2000) Nature Genetics 26 (1):93-6 (PMID: 10973257)
41.Zaki et al. (2007) American Journal Of Medical Genetics. Part A 143A (9):939-44 (PMID: 17431900)
42.Jae et al. (2013) Science (New York, N.Y.) 340 (6131):479-83 (PMID: 23519211)
43.Kumar et al. (2010) Human Molecular Genetics 19 (14):2817-27 (PMID: 20466733)
44.Cushion et al. (2013) Brain : A Journal Of Neurology 136 (Pt 2):536-48 (PMID: 23361065)
45.Ozcelik et al. (2008) Proceedings Of The National Academy Of Sciences Of The United States Of America 105 (11):4232-6 (PMID: 18326629)
46.Boycott et al. (2009) Journal Of Child Neurology 24 (10):1310-5 (PMID: 19332571)
47.Nicholas et al. (2010) Nature Genetics 42 (11):1010-4 (PMID: 20890279)