Prenatal 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]

Lissencephaly may not be seen on ultrasound before 24 weeks of gestation.[4] Other imaging methods, such as 3D ultrasound or fetal MRI, may aid in the diagnosis of lissencephaly in utero.[4,5,6] Due to genetic heterogeneity and overlapping phenotypes, the specific diagnosis cannot be determined accurately with imaging alone. When available, genetic testing can aid in determining the precise diagnosis after the differential has been established by imaging.

Inheritance Pattern/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.[7,8] 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.

Additionally, genotype analysis of maternal and fetal DNA for several polymorphic markers to test for maternal cell contamination will be performed. Therefore, in all prenatal cases a maternal sample should accompany the fetal sample.

Test Sensitivity:
The clinical sensitivity of sequencing and deletion/duplication analysis of the genes included in this panel in prenatal cases ascertained based on fetal ultrasound abnormalities is currently unknown, and the clinical sensitivity of analysis of the genes on the Prenatal Lissencephaly 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[9]
ACTG1	Actin, Gamma-1	AD	20% of Baraitser-Winter syndrome[9]
*ARX**	Aristaless-related homeobox protein	XL	70-95% of XLAG7,8, 3-10% in XLID[12,13]
ATP6V0A2	Lysosomal H(+)-ATPase V0 subunit A2	AR	21-24% of autosomal recessive cutis laxa type II[14,15]
B3GALNT2	Beta-1,3-N-Acetylgalactosaminyltransferase 2	AR	Rare in alpha-dystroglycanopathies[16] Rare in congenital muscular dystrophy[17]
*B4GAT1 (B3GNT1)***	Beta-1,4-Glucuronyltransferase 1	AR	Rare in alpha-dystroglycanopathies[18,19]
CIT	Citron rho-interacting serine/threonine kinase	AR	Rare in MCPH[20,21,22]
DCX	Doublecortin	XL	Up to 100% XL lissencephaly 10% of classic lissencephaly 85% females and ~30% males with SBH [1,11,23]
*FKRP***	Fukutin-related protein	AR	~2% of cobblestone lissencephaly[3,24] 9% of alpha-dystroglycanopathies[25] 6% of limb-girdle MD[26] 2.5% of congenital muscular dystrophy[17]
FKTN	Fukutin	AR	~7% of alpha-dystroglycanopathies[27] Rare in congenital muscular dystrophy[17] 1.5% of limb-girdle MD[28] Does not include the Japanese founder mutation in the 3' UTR[29]
GMPPB	GDP-Mannose Pyrophosphorylase B	AR	Rare in alpha-dystroglycanopathies[30,31] Rare in congenital muscular dystrophy[17]
ISPD	Isoprenoid synthase domain-	AR	~6% of cobblestone

Gene	Protein	Inheritance	Disease Associations
	containing protein		lissencephaly[27] ~30% of Walker-Warburg syndrome and ~11% of alpha-dystroglycanopathies[32,33] Rare in LGMD[34]
KATNB1	Katanin regulatory subunit B1	AR	<1% of MCD[35,36]
LAMB1	Laminin, Beta-1	AR	Rare in cobblestone lissencephaly[37]
LARGE1	Like-glycosyltransferase	AR	2-5% of cobblestone lissencephaly[3,24] ~1% of alpha-dystroglycanopathies[25,27]
NDE1	nudE neurodevelopment protein 1	AR	Rare[38,39,40]
PAFAH1B1 (LIS1)	Platelet-Activating Factor Acetylhydrolase 1b, Regulatory Subunit 1	AD	~40-65% of classic lissencephaly[1,41]
*POMGNT1**	Protein O-Mannose Beta 1-2-N-Acetylglucosaminyltransferase	AR	11-18% of cobblestone lissencephaly[3,24] 8-10% of alpha-dystroglycanopathies[25,27]
POMGNT2	Protein O-Mannose Beta-1,4-N-Acetylglucosaminyltransferase 2	AR	Rare in alpha-dystroglycanopathies[42]
POMT1	Protein O-Mannosyltransferase 1	AR	27-34% of cobblestone lissencephaly[3,24] 9-21% of alpha-dystroglycanopathies[25,27]
POMT2	Protein O-Mannosyltransferase 2	AR	8-11% of cobblestone lissencephaly[3,24] 9-11% of alpha-dystroglycanopathies[25,27]
RELN	Reelin	AR	Rare[43,44]
TMEM5	Transmembrane protein 5	AR	~6% of cobblestone lissencephaly[24] Rare in alpha-dystroglycanopathies[45]
*TUBA1A**	Tubulin, Alpha-1A	AD	1% of classic lissencephaly 30% of lissencephaly with cerebellar hypoplasia[46,47]
VLDLR	Very low density lipoprotein receptor	AR	Rare cerebellar hypoplasia with simplified gyri[48,49]
WDR62	WD repeat-containing protein 62	AR	Unknown[50]

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