Prenatal Pontocerebellar Hypoplasia Panel
Sequence Analysis and Exon-Level Deletion/Duplication Testing of 19 Genes

Panel Gene List: AMPD2, CASK, CHMP1A*, EXOSC3, OPHN1, RARS2, RELN, SEPSECS, TSEN2, TSEN15, TSEN34, TSEN54, TUBA1A*, TUBA8, TUBB2B, TUBB3, VLDLR, VPS53, VRK1
*Only large deletion/duplications may detected for the CHMP1A and TUBA1A genes

Clinical Features:
Pontocerebellar hypoplasia (PCH) is a rare disorder affecting the ventral pons and cerebellum, two structures that share the same neuronal lineage during brain development. PCH has a fetal onset in most cases and appears to result from a combination of a developmental defect and progressive atrophy of the cerebellum.[1-4] Due to the in utero onset and involvement of the pons, PCH can be distinguished from other disorders of abnormal cerebellar development that occur due to prenatal infections, vascular anomalies, degenerative disorders, or metabolic abnormalities. There are three main types of PCH. Type 1 PCH is an infantile-lethal type that affects the anterior horn cells in the spinal cord and causes spinal muscular atrophy, hypotonia, contractures, and microcephaly. Type 2 PCH shows sparing of spinal motor neurons and is characterized by developmental delay, language impairment, dysphagia, progressive microcephaly, and dystonia or chorea. Tonic-clonic seizures, respiratory abnormalities, hypo- or hypertonia, ataxia, and oculomotor abnormalities are also seen in type 2 PCH. Type 4 PCH is similar to but more severe than type 2 PCH, with affected children suffering from contractures, severe generalized clonus, and respiratory failure leading to death in the neonatal period. Other forms of PCH are extremely rare and include variable clinical signs in addition to cerebellar hypoplasia.

In the differential diagnosis for PCH, cerebellar hypoplasia disorders are often considered. These can include X-linked dominant cerebellar hypoplasia disorders without consistent pons involvement that can also present with intellectual disability (XLID), hypotonia, microcephaly, and epilepsy. In addition, autosomal dominant tubulinrelated disorders present with a variety of brain malformations including cerebellar hypoplasia and are caused by abnormal neuronal migration, differentiation, and axonal guidance.[5-7]

Prenatal diagnosis of PCH hypoplasia by ultrasound is very difficult. Other imaging methods, such as 3D ultrasound or fetal MRI, may aid in the diagnosis of PCH in utero.[8,9] 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.[8,9]

Genetics:
The incidence of PCH is not known. This group of disorders manifest as autosomal dominant, recessive or X-linked dominant traits. The neuroradiologic presentation, age of onset, and accompanying clinical signs are often sufficiently distinct to allow clinical classification of the PCH type and correlate with a molecular diagnosis.[1-4] PCH typically manifests as a true Mendelian trait despite the genetic heterogeneity but current literature indicates that clinical heterogeneity can be seen due to pathogenic variants in some genes.

The Pontocerebellar Hypoplasia Panel at GeneDx includes sequencing and deletion/duplication analysis of eighteen genes. These genes encode a variety of proteins, including those involved in microtubule assembly (TUBB genes), components of the transfer RNA splicing protein complex (TSEN genes) and a transfer RNA synthetase responsible for translation of all mitochondrial proteins (RARS2).

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. Alternative sequencing or copy number detection methods are used to analyze regions with inadequate sequence or copy number data. 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 included on the Pontocerebellar Hypoplasia 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.

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: CHMP1A and TUBA1A genes, only whole gene deletions or duplications may be detected

Gene	Protein	Inheritance	Disease Associations
AMPD2	Adenosine monophosphate deaminase 2	AR	Rare[10,11]
CASK	Calcium/calmodulin-dependent serine protein kinase	XL	~4% in cerebellar hypoplasia and intellectual disability [7,12,13]
*CHMP1A**	Charged multivesicular body protein 1A	AR	Rare[14]
EXOSC3	Exosome component 3	AR	~50% of PCH1[15]
OPHN1	Oligophrenin 1	XL	~12% XLID with cerebellar hypoplasia ; ~1% XLID[16]
RARS2	Arginyl-tRNA synthetase 2	AR	Rare[17-19]
RELN	Reelin	AR	Rare[20,21]
SEPSECS	O-phosphoserine tRNA-selenocysteine tRNA synthase	AR	Rare[22,23]
TSEN2	tRNA splicing endonuclease 2	AR	~1-2% of PCH II and IV[24,25]
TSEN15	tRNA splicing endonuclease 2	AR	Rare[26]
TSEN34	tRNA splicing endonuclease 34	AR	~2% of PCH II and IV[24,25]
TSEN54	tRNA splicing endonuclease 54	AR	~60% of PCH (A307S common)[13,19,24,25]
*TUBA1A**	Tubulin, Alpha-1A	AD	~30% of lissencephaly with cerebellar hypoplasia[27,28]
TUBA8	Tubulin, Alpha-8	AR	Rare[29]
TUBB2B	Tubulin, Beta-2B	AD	~3% in cortical malformations including lissencephaly and polymicrogyria[27,30]; ~17% of complex cortical malformations including

			PCH[31]
TUBB3	Tubulin, Beta-3	AD	~10% of complex cortical malformations including PCH[31]
VLDLR	Very low density lipoprotein receptor	AR	Rare cerebellar hypoplasia with simplified gyri[32,33]
VPS53	Vacuolar protein sorting 53	AR	Rare[34]
VRK1	Vaccinia-related kinase 1	AR	Rare[35]

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