Prenatal Pontocerebellar Hypoplasia Panel
Sequence Analysis and Exon-Level Deletion/Duplication Testing of 18 Genes

Panel Gene List: CASK, CHMP1A, CLP1, EXOSC3, OPHN1, RARS2, RELN, SEPSECS, TSEN2, TSEN34, TSEN54, TUBA1A, TUBA8, TUBB2B, TUBB3, VLDLR, VPS53, VRK1

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. 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 tubulin-related 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. Currently, 9 recognized genetic causes specifically account for PCH types 1-6, 8-10, and for closely overlapping cerebellar hypoplasia disorders. 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, coding exons and flanking splice junctions of the genes on this panel are enriched using a proprietary targeted capture method developed by GeneDx. The products are sequenced on an Illumina instrument using paired end reads. The sequence data is aligned to reference sequences based on human genome build GRCh37/UCSC hg19. Sanger sequencing is used to compensate for low coverage and refractory amplifications. Concurrently, targeted array CGH analysis with exon-level resolution is performed to evaluate for a deletion or duplication of one or more exons for the genes included on the panel. The presence of any potentially disease-associated sequence variant(s) or copy number alteration(s) is confirmed by dideoxy DNA sequence analysis or quantitative PCR, respectively, or by other appropriate methods.

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 18 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 the sequencing test is estimated to be 98%. Deletions involving more than 20 bp and insertions involving more than 10 bp are not reliably detected by the sequencing methodology, and deletions or duplications of less than 500 bp are not reliably detected by array CGH. Note that small sections of a few genes have inherent sequence properties that result in suboptimal data and variants in those regions may not be reliably identified.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Inheritance</th>
<th>Disease Associations</th>
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<tbody>
<tr>
<td>CASK</td>
<td>Calcium/calmodulin-dependent serine protein kinase</td>
<td>XL</td>
<td>~4% in cerebellar hypoplasia and intellectual disability&lt;sup&gt;7,10,11&lt;/sup&gt;</td>
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<tr>
<td>CHMP1A</td>
<td>Charged multivesicular body protein 1A</td>
<td>AR</td>
<td>Rare&lt;sup&gt;12&lt;/sup&gt;</td>
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<tr>
<td>CLP1</td>
<td>Cleavage and polyadenylation factor 1 subunit 1</td>
<td>AR</td>
<td>Rare&lt;sup&gt;13,1&lt;/sup&gt;</td>
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<tr>
<td>EXOSC3</td>
<td>Exosome component 3</td>
<td>AR</td>
<td>~50% of PCH1&lt;sup&gt;15&lt;/sup&gt;</td>
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<tr>
<td>OPHN1</td>
<td>Oligophrenin 1</td>
<td>XL</td>
<td>~12% in XLID with cerebellar hypoplasia; ~1% in XLID&lt;sup&gt;16&lt;/sup&gt;</td>
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<tr>
<td>RARS2</td>
<td>Arginyl-tRNA synthetase 2</td>
<td>AR</td>
<td>Rare&lt;sup&gt;17-19&lt;/sup&gt;</td>
</tr>
<tr>
<td>RELN</td>
<td>Reelin</td>
<td>AR</td>
<td>Rare&lt;sup&gt;20,21&lt;/sup&gt;</td>
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<tr>
<td>SEPSECS</td>
<td>O-phosphoserine tRNA-selenocysteine tRNA synthase</td>
<td>AR</td>
<td>Rare&lt;sup&gt;22,23&lt;/sup&gt;</td>
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<tr>
<td>TSEN2</td>
<td>tRNA splicing endonuclease 2</td>
<td>AR</td>
<td>~1-2% of PCH II and IV&lt;sup&gt;24,25&lt;/sup&gt;</td>
</tr>
<tr>
<td>TSEN34</td>
<td>tRNA splicing endonuclease 34</td>
<td>AR</td>
<td>~2% of PCH II and IV&lt;sup&gt;24,25&lt;/sup&gt;</td>
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<tr>
<td>TSEN54</td>
<td>tRNA splicing endonuclease 54</td>
<td>AR</td>
<td>~60% of PCH (A307S common)&lt;sup&gt;11,19,24,25&lt;/sup&gt;</td>
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<tr>
<td>TUBA1A</td>
<td>Tubulin, Alpha-1A</td>
<td>AD</td>
<td>30% of lissencephaly with cerebellar hypoplasia&lt;sup&gt;26,27&lt;/sup&gt;</td>
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<tr>
<td>TUBA8</td>
<td>Tubulin, Alpha-8</td>
<td>AR</td>
<td>Rare&lt;sup&gt;28&lt;/sup&gt;</td>
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<tr>
<td>TUBB2B</td>
<td>Tubulin, Beta-2B</td>
<td>AD</td>
<td>~3% in cortical malformations including lissencephaly and polymicrogyria&lt;sup&gt;26,29&lt;/sup&gt; ~17% of complex cortical malformations&lt;sup&gt;30&lt;/sup&gt;</td>
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<tr>
<td>TUBB3</td>
<td>Tubulin, Beta-3</td>
<td>AD</td>
<td>~10% of complex cortical malformations, including PCH&lt;sup&gt;31&lt;/sup&gt;</td>
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<td>VLDLR</td>
<td>Very low density lipoprotein receptor</td>
<td>AR</td>
<td>Rare cerebellar hypoplasia with simplified gyri&lt;sup&gt;32,33&lt;/sup&gt;</td>
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<tr>
<td>VPS53</td>
<td>Vacuolar protein sorting 53</td>
<td>AR</td>
<td>Rare&lt;sup&gt;34&lt;/sup&gt;</td>
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<tr>
<td>VRK1</td>
<td>Vaccinia-related kinase 1</td>
<td>AR</td>
<td>Rare&lt;sup&gt;35&lt;/sup&gt;</td>
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References:
