THE UNIVERSITY OF CHICAGO | Genetic Services Laboratories

Next Generation Sequencing Panel for Cerebellar Hypoplasia

Pontocerebellar Hypoplasia

Pontocerebellar hypoplasia (PCH) is a group of rare autosomal recessive neurodegenerative disorders with a prenatal onset, characterized by cerebellar hypoplasia in addition to varying degrees of atrophy of the cerebellum and pons [1]. MRI findings include a small cerebellum and brainstem, variable neocortical atrophy, severe and progressive microcephaly and variable ventriculomegaly [1]. Clinically, most patients have severe intellectual disability, swallowing problems, and seizures.

Cerebellar Vermis Hypoplasia

Cerebellar Vermis Hypoplasia (CVH) consists of isolated vermis hypoplasia and may also be called "Dandy-Walker variant" due to the phenotypic overlap with Dandy-Walker malformation (DWM). DWM includes vermis hypoplasia in addition to several other features such as enlarged posterior fossa.

Our Cerebellar/Pontocerebellar Hypoplasia Panel includes analysis of the 27 genes listed below.

Cerebellar/Pontocerebellar Hypoplasia Panel Genes			
AMPD2	OPHN1	TBC1D23	TUBA8
CASK	PCLO	TOE1	TUBB2B
CDK5	RAB11B	TSEN2	TUBB3
CHMP1A	RARS2	TSEN15	VLDLR
CLP1	RELN	TSEN34	VPS53
EXOSC3	SEPSECS	TSEN54	VRK1
EXOSC8	SON	TUBA1A	

Disorder and Associated Genes	Clinical Features / Molecular Pathology
PCH type 1 [OMIM#607596] PCH type 1B [OMIM#614678] PCH type 1C [OMIM#616081] *VRK1* [OMIM#602168] *EXOSC3* [OMIM#606489] *EXOSC8* [OMIM#606019]	PCH type 1 is characterized by loss of motor neurons in the spinal cord, which is morphologically similar to the hereditary spinal muscular atrophies, in addition to the typical findings of PCH [1]. Renbaum *et al.* (2009) identified a homozygous nonsense mutation in *VRK1* in a consanguineous family with PCH type 1. *VRK1* encodes a serine-threonine kinase which is thought to play a role in nervous system development and neuronal maintenance [2]. Wan *et al*, (2012) identified homozygous and compound heterozygous mutations in *EXOSC3* in affected members of nine families with PCH type 1B [3]. EXOSC3 is a core component of the human RNA exosome complex. Boczonadi *et al.* (2014) identifed homozygous *EXOSC8* mutations in 22 infants from three families with cerebellar and corpus callosum hypoplasia, abnormal CNS myelination or spinal motor neuron disease. *EXOSC8* is a core component of the human RNA exosome complex [4].
PCH type 2 [OMIM#277470] *SEPSECS* [OMIM#613009] *TSEN54* [OMIM#608755] *TSEN34* [OMIM#608755] *TSEN15* [OMIM#608756] *TSEN2* [OMIM#608753] *VPS53* [OMIM#615850]	PCH type 2 is characterized by dyskinesia and dystonia and is the most common subtype of PCH [5]. Mutations in *TSEN54, TSEN2* and *TSEN34* are associated with PCH type 2. *TSEN54* encodes one of the noncatalytic subunits of the tRNA splicing endonuclease complex, and *TSEN2* and *TSEN34* encode catalytic subunits of the tRNA splicing endonuclease. This complex has a high abundance of its mRNA in the developing pons, cerebellar dentate and olivary nuclei, suggesting it is importance for the development of these brain areas. Budde *et al.* (2008) sequenced the *TSEN54, TSEN2 and TSEN34* genes in 52 patients with PCH type 2, and identified a common *TSEN54* missense mutation (p.A307S) in the homozygous state in 47/52 patients, a homozygous missense mutation in *TSEN2* one patient, and a homozygous missense mutation in *TSEN34* in one other patient [6]. Homozygous or compound heterozygous missense mutations in the *SEPSECS* gene have been identified in 4 unrelated patients of Iraqi or Iraqi/Moroccon descent with cerebellocerebral atrophy, profound intellectual disability and spasticity most consistent with pontocerebellar hypoplasia type 2 [6]. Homozygous mutations in *TSEN15* have been reported in patients with PCH type 2F from consanguineous families [7,8] Compound heterozygous variants in affected individuals with PCH type 2E of Moroccan Jewish

	ancestry [9].
PCH type 3 [OMIM#608027] PCLO [OMIM#604918]	A homozygous truncating variant in the PCLO gene has been described in a consanguineous family with multiple affected individuals with pontocerebellar hypoplasia [10].
PCH type 4 [OMIM#225753] *TSEN54* [OMIM#608755]	PCH type 4, also known as fatal infantile olivopontocerebellar hypoplasia, has clinical overlap with PCH type 2, however it has a more severe course and is often associated with early postnatal death [6]. The findings of polyhydramnios and contractures have been described prenatally in some cases of PCH type 4 [1]. Budde *et al.* (2008) sequenced the *TSEN54* gene in 3 patients with PCH type 4, and identified homozygous mutations in all patients [6].
PCH type 6 [OMIM#611523] *RARS2* [OMIM#611524]	Characteristic features of PCH type 6 include cerebral atrophy, hypotonia, convulsions and multiple respiratory chain defects [6]. Edvardson *et al.* (2007) identified an intronic mutation in the *RARS2* gene in a consanguineous family with PCH type 6 [11]. *RARS2* enocodes for a mitochondrial arginine tRNA synthetase and plays a role in protein synthesis and tRNA processing, however the underlying mechanism of disease is not well understood [1]. Namavar *et al.* (2011) identified 2 patients with *RARS2* mutations out of a cohort of 169 patients referred for molecular testing for PCH of varying subtypes.
PCH type 7 [OMIM#614969] *TOE1* [OMIM#613931]	Biallelic mutations in the *TOE1* gene have been observed in mutliple unrelated families with PCH type 7 [12].
PCH type 8 [OMIM#614961] *CHMP1A* [OMIM#164010]	PCH type 8 is characterized by severe psychomotor retardation, abnormal movements, hypotonia, spasticity and variable visual defects. Brain MRI show pontocerebellar hypoplasia, decreased cerebral white matter and a thin corpus callosum. Mochida *et al* (2012) identified 2 different homozygous mutations in *CHMP1A* in families with PCH type 8 [13].
PCH type 9 [OMIM#615809] *AMPD2* [OMIM#102771]	In 5 families with pontocerebellar hypoplasia type 9, Akizu *et al.* (2013) identified 5 different homozygous mutations in the AMPD2 gene [14]. Brain MRI showed a unique finding which was present on axial images in which the brainstem takes on a "figure 8" appearance, with relative preservation of the cerebellar vermis. There was also generalized atrophy of the cerebral cortex and severe corpus callosum hypoplasia. Mutations causing premature truncation as well as missense mutations of highly conserved amino acid residues have been reported.
PCH type 10 [OMIM#615803] *CLP1* [OMIM#608757]	The same homozygous missense mutation, R140H, was identified in affected family members of 9 consanguineous Turkish families. Haplotype analysis indicated this change as a founder mutation. Affected individuals presented with motor-sensory defects, microcephaly, spacticity, seizures and inability to walk. Imaging of the brain showed variable abnormaltieis including cortical dysgenesis, pontocerebellar atrophy or hypoplasia, and thin corpus callosum. In vitro functional studies suggest that the mutation causes loss of tRNA splicing endonuclease (TSEN) complex function, resulting in accumulation of unprocessed pre-tRNAs [15,16].
PCH type 11 [OMIM#617695] TBC1D23 [OMIM#617687]	Biallelic mutations in the *TBC1D23* gene have been observed in mutliple unrelated families with PCH type 11 [17,18].
X-linked mental retardation and microcephaly with pontine and cerebellar hypoplasia (MIC-PCH) [OMIM#300749] *CASK* [OMIM#300172]	MIC-PCH is associated with mutations in the *CASK* gene and is characterized by severe or profound intellectual disability and structural brain anomalies including congenital progressive microcephaly, simplified gyral pattern, thin brain stem with flattening of the pons, and severe cerebellar hypoplasia in females [19]. Seizures, sensorineural hearing loss and retinal anomalies (optic disk pallor/optic nerve hypoplasia) may also be present. Mutations associated with MIC-PCH are typically *de novo* and are thought to be lethal in males [20]. Milder, familial mutations have also been described that are associated with mild to moderate intellectual disability in males, and no symptoms in carrier females [20]. *CASK* encodes a calcium/calmodulin-dependent serine protein kinase and functions in both pre- and post-synaptic sites as part of large signaling complexes. Tarpey *et al.* (2009) identified *CASK* mutations in 4/46 individuals with MIC-PCH [21].
X-linked Mental Retardation with Cerebellar Hypoplasia [OMIM #300486] *OPHN1* [OMIM#300127]	Mutations in the *OPHN1* gene have been identified in patients with X-linked Mental Retardation with Cerebellar Hypoplasia [22,23]. In patients with *OPHN1* mutations, magnetic resonance imaging (MRI) may also reveal cerebral atrophy and ventriculomegaly. Physical findings typically include tall stature, macrocephaly, and common facial features such as deep-set eyes, long tubular nose, short philtrum, thin upper lip and prominent chin. Other features may include seizures, oculomotor problems, dysmetria, adiadochokinesia, hyperactivity, and anxiety. Most heterozygous females have mild cognitive handicaps [22,23]. *OPHN1* is highly expressed in fetal brain tissue and is postulated to affect cell migration and outgrowth of axons and dendrites [6]. Philip *et al* (2003) reported that 2/6 (33%) males with moderate mental retardation and cerebellar vermis hypoplasia had mutations in *OPHN1* [22].

TUBA1A [OMIM#602529] *TUBA8* [OMIM#605742] *TUBB2B* [OMIM#612850] *TUBB3* [OMIM#602661]	The tubulin-related cortical dysgeneses are though to involve a combination of abnormal neuronal proliferation, migration, differentiation and axonal guidance [24]. Cerebellar vermian hypoplasia is a prominent feature of *TUBA1A*-related disorders and is also identified in patients with *TUBA8*, *TUBB2B* and *TUBB3*-related disorders [25].
CDK5 [OMIM# 616342]	In a consanguineous family with individuals affected by severe lissencephaly, cerebellar hypoplasia and agenesis of the corpus callosum, Magen *et al* (2015) identified a homozygous truncating variant in the *CDK5* gene [26].
RAB11B [OMIM#604198]	*De novo* variants in *RAB11B* have been observed in patients with a distinct brain phenotype, consisting of abnormal white matter, thin corpus callosum, cerebellar hypoplasia, optic nerve hypoplasia and ventriculomegaly. Affected individuals typically have a neurodevelopmental phenotype, including severe intellectual disability with absent speech, epilepsy, and hypotonia, visual problems, musculoskeletal abnormalities, and microcephaly [27].
RELN [OMIM#600514] *VLDLR* [OMIM#192977]	*RELN* mutations have been identified in patients with a less severe form of lissencephaly with cerebellar hypoplasia (LCH) [28]. *VLDLR*-associated cerebellar hypoplasia (*VLDLR*-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. *VLDR* is part of the reelin (*RELN*) signaling pathway, which guides neuroblast migration in the cerebral cortex and cerebellum. LCH is distinguished from *VLDLR*-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 [29].
ZTTK syndrome [OMIM#617140] *SON* [OMIM#182465]	*De novo* variants in the *SON* gene are associated with ZTTK syndrome, which is characterized by D and/or DD, malformations of the cerebral cortex, epilepsy, vision problems, musculoskeletal abnormalities, and congenital malformations. 89% of affected patients have abnormalities identified on brain MRI including abnormal gyral patterns, ventriculomegaly, arachnoid cysts, hypoplasia of the corpus callosum, cerebellar hypoplasia, and loss of periventricular white matter [30].

Inheritance:

AMPD2, CDK5, CHMP1A, CLP1, EXOSC3, EXOSC8, PCLO, RARS2, RELN, SEPSECS, TBC1D23, TOE1, TSEN15, TSEN2, TSEN34, TSEN54, TUBA8, VLDLR and *VPS53, VRK1* mutations are inherited in an autosomal recessive pattern. Parents of an affected child are most likely obligate carriers. Recurrence risk for carrier parents is 25%.

RAB11B, SON, TUBA1A, TUBB2B and *TUBB3* mutations are inherited in an autosomal dominant pattern. All *TUBB2B* mutations described to date have been *de novo* in nature. The recurrence risk for parents is less than 1%, based on the theoretical risk for germline mosaicism. Both *de novo* and inherited mutations in *TUBB3* have been described. The recurrence risk for unaffected parents of an isolated case is <1%. The recurrence risk for affected parents is 50%.

CASK mutations associated with MIC-PCH are typically *de novo* in females and thought to be lethal in males. Recurrence risk for parents of an affected child is <1% for *de novo* mutations, based on the risk of gonadal mosaicism. Milder *CASK* mutations can be associated with mild to moderate intellectual disability in males, and carrier females may be asymptomatic. Recurrence risk for a carrier female is 50% in a male child.

Mutations in *OPHN1* are inherited in an X-linked pattern and result in clinical features in affected males and females. Males are more severely affected than females. A woman who has more than one affected son is an obligate carrier. Recurrence risk for carrier mothers is 50%.

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.

Our CNV detection algorithm was developed and its performance determined for the sole purpose of identifying deletions and duplications within the coding region of the gene(s) tested. Partial exonic copy number changes and rearrangements of less than 400 bp may not be detected by this methodology. Regions of high homology and repetitive regions may not be analyzed. This methodology will not detect low level mosaicism, balanced translocations, inversions, or point mutations that may be responsible for the clinical phenotype. The sensitivity of our deletion/duplication assay may be reduced when DNA extracted by an outside laboratory is provided.

Cerebellar/Pontocerebellar Hypoplasia Panel (27 genes)

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	$2800
CPT codes:	81406 81407
Turn-around time:	8 weeks

Note: We cannot bill insurance for the above test.

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 or contact us at 773-834-0555.

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