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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 Hypoplasia Panel includes all seven genes listed below.

Cerebellar Hypoplasia			
VRK1	TSEN54	RARS2	OPHN1
TSEN2	TSEN34	CASK	

Disorder and Associated Genes	Clinical Features / Molecular Pathology
PCH type 1 [OMIM#607596] *VRK1* [OMIM#602168]	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].
PCH type 2 [OMIM#277470] *TSEN54* [OMIM#608755] *TSEN34* [OMIM#608755] *TSEN2* [OMIM#608753]	PCH type 2 is characterized by dyskinesia and dystonia and is the most common subtype of PCH [3]. 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.
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 [4]. 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 [4].
PCH type 6 [OMIM#611523] *RARS2* [OMIM#611524]	Characteristic features of PCH type 6 include cerebral atrophy, hypotonia, convulsions and multiple respiratory chain defects [4]. Edvardson *et al.* (2007) identified an intronic mutation in the *RARS2* gene in a consanguineous family with PCH type 6. *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.

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 [6]. 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 [7]. Milder, familial mutations have also been described that are associated with mild to moderate intellectual disability in males, and no symptoms in carrier females [7]. *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.
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 [9,10]. 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 [9,10]. *OPHN1* is highly expressed in fetal brain tissue and is postulated to affect cell migration and outgrowth of axons and dendrites [4]. Philip *et al* (2003) reported that 2/6 (33%) males with moderate mental retardation and cerebellar vermis hypoplasia had mutations in *OPHN1*.

Inheritance:

VRK1, TSEN54, TSEN2, TSEN34 and *RARS2* 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%.

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 amplified using highly parallelized and multiplexed PCR reactions assembled with the Raindance System. DNA is sequenced 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 novel and/or potentially 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 8 bp.

Cerebellar Hypoplasia Next Generation Sequencing Panel

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	$3975
CPT codes:	83891, 83900, 83901 X14, 83904 X13, 83912
Turn-around time:	8 – 10 weeks

Note: The sensitivity of our assay may be reduced when DNA is extracted by an outside laboratory. Only fresh blood samples are accepted for this testing.

Testing for a known mutation in additional family members by sequence analysis

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	$390
CPT codes:	83891, 83898 x2, 83894, 83912
Turn-around time:	3-4 weeks

Prenatal testing for a known mutation by sequence analysis

Sample specifications:	2 T25 flasks of cultured cells from amniocentesis or CVS or 10 mL of amniotic fluid
Cost:	$540
CPT codes:	83891, 83898 x4, 83894, 83912
Turn-around time:	1-2 weeks

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.

References:

1. Namavar Y *et al*., "Clinical, neuroradiological and genetic findings in pontocerebellar hypoplasia." (2011) Brain 134: 143-156.
2. Renbaum P *et al*., "Spinal Muscular Atrophy with Pontocerebellar Hypoplasia is Caused by a Mutation in the VRK1 gene." (2009) Am J Hum Genet 85: 281-89.
3. Namavar Y, et al. "Classification, diagnosis, and potential mechanisms in Pontocerebellar Hypoplasia". (2011) Orphanet Journal of Rare Diseases 6: 50.
4. Budde BS, et al. "tRNA splicing endonuclease mutations cause pontocerebellar hypoplasia". (2008) Nature Genet 40: 1113-1118.
5. Edvardson S et al., "Deleterious mutation in the mitochondrial arginyl-transfer RNA synthetase gene is associated with pontocerebellar hypoplasia." (2007) Am J Hum Genet 81: 857-62.
6. Namavar Y *et al*., "Pontocerebellar Hypoplasia Type 2 and Type 4." GeneReviews, last updated Sept. 2009.
7. Najm J, *et al*. "Mutations of CASK cause an X-linked brain malformation phenotype with microcephaly and hypoplasia of the brainstem and cerebellum". (2008) Nature Genet. 40: 1065-1067.
8. Tarpey P. S., *et al*. "A systematic, large-scale resequencing screen of X-chromosome coding exons in mental retardation". (2009) Nature Genet. 41: 535-543.
9. Philip N, et al. "Mutations in the oligophrenin-1 gene (OPHN1) cause X-linked congenital cerebellar hypoplasia". (2003) J Med Genet 40:441-446.
10. Zanni G, et al. "Oligophrenin 1 mutations frequently cause X-linked mental retardation with cerebellar hypoplasia". (2005) Neurology 65: 1364-69.

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