THE UNIVERSITY OF CHICAGO | Genetic Services Laboratories

Next Generation Sequencing Panel for Holoprosencephaly

Clinical Features:
Holoprosencephaly (HPE) is a very common malformation of forebrain development and is defined as the incomplete separation of the two cerebral hemispheres [1]. HPE can be further subdivided based on the severity of the defect: alobar HPE, semilobar HPE and lobar HPE. Facial abnormalities occur in approximately 80% of HPE patients and can include cyclopia, proboscis and cleft lip/palate in the severe form, to a single central maxillary incisor, ocular hypotelorism and nasal abnormalities in the more mild forms [1]. HPE occurs in 1 in 120 fetuses and 1 in 16,000 live births. Cytogenetically visible anomalies are estimated to be present in approximatey 25% of patients with HPE, while mutations are estimated to be identifiable in approximately 17% of cytogenetically normal HPE patients [2].

Our Holoprosencephaly Panel includes mutation analysis of the 10 genes listed below.

Holoprosencephaly Sequencing Panel				
CDON	FGFR1	PTCH1	SIX3	TGIF1
FGF8	GLI2	SHH	STIL	ZIC2

Gene	Clinical Features	Details
CDON	Holoprosencephaly 11	Mutations in *CDON* have been identified in four unrelated individuals with holoprosencephaly spectrum disorders [3]. Clinical features included agenesis of the corpus callosum and alobar HPE.
FGF8	Holoprosencephaly, craniofacial defects, and hypothalamo-pituitary dysfunction	A homozygous missense mutation in *FGF8* was recently identified in a consanguineous patient with semilobar HPE, diabetes insipidus, and TSH and ACTH insufficiency [2]. Heterozygous mutations in *FGF8* have also been identified in patients with Kallman syndrome
FGFR1	Hartsfield syndrome	Homozygous and heterozygous mutations in *FGFR1* have been identified in a small number of patients with Hartsfield syndrome [4]. The clinical characteristics of Hartsfield syndrome include holoprosencephaly, ectrodactyly and cleft lip/palate. Profound intellectual disability and multiple congenital anomalies may also be present.
GLI2	Holoprosencephaly 9	Mutations in *GLI2* have been identified in patients with holoprosencephaly 9, which is characterized by a wide phenotypic spectrum of brain developmental defects with or without overt forebrain cleavage abnormalities [5]. This disorder shows incomplete penetrance and variable expressivity.
PTCH1	Holoprosencephaly 7	Mutations in *PTCH1* are a rare cause of holoprosencephaly. One study identified four heterozygous missense mutations in PTCH1 in 5 out of 60 patients with holoprosencephaly or a holoprosencephaly-like disorder [6].
SHH	Holoprosencephay 3	Mutations in *SHH* account for approximately 6-8% of patients with holoprosencephaly or a holoprosencephaly-like disorder [7]. Intrafamilial variability and incomplete penetrance has been noted and phenotypes of *SHH* patients can range from cyclopia to the less severe solitary median maxillary central incisor.
SIX3	Holoprosencephaly 2	Mutations in *SIX3* have been identified in 4 to 10% (depending on the study) patients with Holoprosencephaly 2 [8]. Missense, nonsense and frameshift mutations have all been reported. There are no significant differences in the clinical features of patients with either missense changes or truncating changes, and generally speaking *SIX3* mutations result in a more severe phenotype than other gene mutations for holoprosencephaly.
STIL	Autosomal recessive primary microcephaly-7	Kaker *et al* (2015) reported a homozygous truncating mutation in the STIL gene in a family with severe microcephaly and lobar holoprosencephaly [9]. Affected family members had severe intellectual disability, but no cleft lip or palate, and no single central maxillary incisor.

TGIF1	Holoprosencephaly 4	Mutations in *TGIF1* have been identified in approximately 1-2% of patients with holoprosencephaly [10]. Missense, nonsense and entire gene deletions due to cytogenetically visible rearrangements have all been reported. Phenotypically, clinical features run the gamut.
ZIC2	Holoprosencephaly 5	Mutations in *ZIC2* have been identified in up to 10% of patients with holoprosencephaly [10]. Some studies have postulated that facial malformations in *ZIC2* patients are less apparent than in patients with mutations in the other genes.

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.

Holoprosencephaly Panel (10 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.

References:

1. Geng, X. and G. Oliver, *Pathogenesis of holoprosencephaly.* J Clin Invest, 2009. **119**(6): p. 1403-13.
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3. Bae, G.U., et al., *Mutations in CDON, encoding a hedgehog receptor, result in holoprosencephaly and defective interactions with other hedgehog receptors.* Am J Hum Genet, 2011. **89**(2): p. 231-40.
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6. Ribeiro, L.A., J.C. Murray, and A. Richieri-Costa, *PTCH mutations in four Brazilian patients with holoprosencephaly and in one with holoprosencephaly-like features and normal MRI.* Am J Med Genet A, 2006. **140**(23): p. 2584-6.
7. Roessler, E., et al., *The mutational spectrum of holoprosencephaly-associated changes within the SHH gene in humans predicts loss-of-function through either key structural alterations of the ligand or its altered synthesis.* Hum Mutat, 2009. **30**(10): p. E921-35.
8. Ribeiro, L.A., et al., *SIX3 mutations with holoprosencephaly.* Am J Med Genet A, 2006. **140**(23): p. 2577-83.
9. Kakar, N., et al., *STIL mutation causes autosomal recessive microcephalic lobar holoprosencephaly.* Hum Genet, 2015. **134**(1): p. 45-51.
10. Keaton, A.A., et al., *TGIF Mutations in Human Holoprosencephaly: Correlation between Genotype and Phenotype.* Mol Syndromol, 2010. **1**(5): p. 211-222.

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