Comprehensive Holoprosencephaly Panel
Sequence Analysis and Exon-Level Deletion/Duplication Testing of 17 Genes

Panel Gene List: *CDON, DISP1, DLL1, FGF8, FGFR1, FOXH1, GLI2, GAS1, NODAL, PTCH1, SHH, SIX3, SMAD2, STIL, TDGF1, TGIF1, ZIC2*

Clinical Features:
Holoprosencephaly (HPE) is the most common malformation of the forebrain and midface, affecting 1 in 250 gestations, and 1 in 10,000 live-born infants. Cental nervous system (CNS) abnormalities result from the failure of the forebrain to separate into right/left lobes and can be labeled as alobar, semiloar, or lobar holoprosencephaly. Milder CNS abnormalities involving fusion of more limited brain structures include middle interhemispheric fusion variant/ syntelencephaly and septopreoptic types.1 About 80% of individuals with HPE have characteristic facial anomalies ranging from hypotelorism and single central upper incisor (microform) to cyclopia with proboscis. Neurocognitive impairment, seizures, cleft lip/palate, skeletal anomalies, congenital heart defects, autonomic instability, diabetes insipidus and other endocrine abnormalities also constitute frequent complications of HPE.2 Developmental delay is seen in all individuals with an HPE-associated brain anomaly.

Genetics:
Chromosomal abnormalities are identified in up to 50% of individuals with HPE, including trisomy of chromosomes 13 / 18, triploidy, and various translocations or deletions/duplications. Another 25% of patients with HPE have a recognizable genetic syndrome (e.g. Pallister-Hall syndrome, Smith-Lemli-Opitz syndrome, Hatsfield syndrome, etc).[1,3,4] The remainder of patients are classified as having non-syndromic HPE.

The majority of non-syndromic HPE is caused by variants in the SHH, SIX3, TGIF1, and ZIC2 genes. Autosomal dominant inheritance with reduced penetrance and significant variable expressivity, even within the same family, is expected. Multiple genes within the sonic hedgehog and NOTCH/NODAL signaling pathways are associated with a smaller number of individuals with non-syndromic HPE due to their functional importance in early embryonic patterning.[5] Rarely HPE families with a complex, recessive inheritance have been reported, the proband having a variant in each of two different HPE-associated genes, and each parent being a clinically normal carrier for one of the variants. Germline mosaicism has been observed.

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. Multiplex ligation-dependent probe amplification (MLPA) is used to detect deletions and duplications involving the SHH, SIX3, ZIC2, TGIF, and GLI2 genes. 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.

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: GAS1 and SMAD2 gene, no copy number testing, FGF8 gene, only whole gene deletions or duplications may be detected.

Clinical Sensitivity:
Of patients with nonsyndromic HPE, approximately 15% will have a reportable sequencing or copy number variant in the SHH, SIX3, TGIF1 or ZIC2 genes.6,7 These autosomal dominant variants typically express reduced penetrance and variable expressivity. The full variant spectrum includes both microdeletions and missense, nonsense, and frameshift sequencing variants.

Gene	Inheritance	Disease Phenotype Associations	Diagnostic Yield for Disorder in Postnatal Populations
CDON	AD	HPE Type 11, SHH pathway	Rare [8]
DISP1	AD	HPE Type 10, HPE-like Microform type	Rare [9]
DLL1	AD	Nodal pathway	Rare [9]
FGF8	AR	HPE, craniofacial defects, and hypothalamo-pituitary dysfunction, Kallman syndrome	Unknown [9]

Gene	Inheritance	Disease Phenotype Associations	Diagnostic Yield for Disorder in Postnatal Populations
FGFR1	AD / AR	HPE, ectrodactyly, and cleft lip/palate, Hartsfield Syndrome	Unknown [9]
FOXH1	AD	HPE, cardiac defects, Nodal pathway	Rare [10]
GAS1	AD	Classic HPE spectrum, SHH pathway	Rare [11, 12]
GLI2	AD	HPE Type 9, HPE, hypopituitarism, polydactyly, Culler-Jones syndrome	Unknown [9,13, 14]
NODAL	AD	HPE, cardiac, laterality defects	Rare [15]
PTCH1	AD	HPE Type 7, SHH pathway, classic HPE with high intra-familial variability. (Haploinsufficiency also causes Gorlin syndrome)	Rare [16, 17]
SHH	AD	HPE Type 3, Schizencephaly, Microphthalmia, higher number of familial variants & intra-familial variability, microform variants	6% of non-syndromic HPE [6,7]
SIX3	AD	HPE Type 2, Schizencephaly, higher number of familial variants & intra-familial variability	3% of non-syndromic HPE [6,7]
SMAD2	AD	HPE, cardiac defects, Nodal pathway	Rare [10]
STIL	AR	Primary Microcephaly	Rare [18]
TDGF1	AD	HPE, forebrain defects	Rare [19]
TGIF1	AD	HPE Type 4	<1% of non-syndromic HPE [6]
ZIC2	AD	HPE Type 5, milder facial characteristics, severe CNS involvement, higher number of de novo variants, higher penetrance	5% of non-syndromic HPE [6,7]

Abbreviations: AD – Autosomal Dominant, AR – Autosomal Recessive, HPE – Holoprosencephaly

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