Genetic Testing in Holoprosencephaly

Clinical Features:
Holoprosencephaly (HPE) is the most common malformation of the forebrain and midface. It is characterized by a wide phenotypic spectrum ranging from hypotelorism and single central upper incisor to cyclopia with proboscis. About 80% of individuals with HPE have characteristic facial anomalies. Neurocognitive impairment, seizures, cleft lip/palate, autonomic instability, diabetes insipidus and other endocrine abnormalities constitute frequent complications of HPE. HPE affects 1 in 250 gestations, and 1 in 10,000 live-born infants.

Genetics:
The disorder follows a complex pattern of inheritance combining multiple genetic and environmental factors. In families with an identified variant in one of the known HPE genes, approximately half of the variants occur de novo. Autosomal dominant inheritance, with reduced penetrance and variable expressivity is common. Rarely, have HPE families with a complex, recessive inheritance 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 obtained from the submitted specimen, bi-directional DNA sequence of the coding regions and flanking splice sites is obtained and analyzed for all or a portion of four genes associated with HPE. The entire coding regions of the TGIF and SIX3 genes are evaluated, and all but the sequence corresponding to the last 120 codons of the ZIC2 gene and the last 50 codons of the SHH gene, including the stop codon, are evaluated. Concurrently, multiplex ligation-dependant probe amplification (MLPA) is performed to evaluate for deletions or duplications of one or more exons of these genes.

Test Sensitivity, Genotype-Phenotype Correlation, Variant Spectrum:
In approximately 18-25% of affected individuals, HPE is part of a recognizable genetic syndrome (e.g. Pallister-Hall syndrome, Smith-Lemli-Opitz syndrome, or others). Up to 50% of HPE cases are due to chromosomal abnormalities, including trisomy of chromosomes 13 or 18 and various other structural changes.

Of patients with nonsyndromic HPE, approximately 13-25% will have a variant in the SHH, TGIF, SIX3, or ZIC2 gene identifiable by DNA sequencing. Specifically, of patients with HPE, an estimated 12% have a variant in the SHH2,[9], 5% in SIX3 [8], 8% in ZIC210, and 1-2% have a variant in the TGIF gene.[5,6] Such variants are usually inherited in an autosomal dominant manner. The variant spectrum includes missense, nonsense, and frameshift variants. The

methods used by GeneDx would not identify variants in certain portions of these genes (see Test Method section); however, fewer than 20% of variants in ZIC2 or SHH have been found in the regions not analyzed. The sensitivity of HPE sequence analysis in prenatal cases ascertained based on fetal ultrasound abnormalities is currently unknown.

Microdeletions of the SHH, TGIF, SIX3, or ZIC2 genes have also been identified in patients with holoprosencephaly. In one recent study, 16 of 339 (4.7%) patients with severe holoprosencephaly, a normal karyotype, and no point variants by sequencing had a microdeletion of one of the four genes.[4] Additionally, microdeletions were identified in 8 of 94 (8.5%) fetuses with a normal karyotype and no point variants. [4]

The phenotype associated with pathogenic variants in the nonsyndromic HPE genes is extremely variable. Patients with ZIC2 variants may have milder facial characteristics, despite severe CNS involvement. Pathogenic variants are identified in the SHH gene more frequently than variants in other HPE genes and are present in 30-40% of cases of autosomal dominant HPE.

References:

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