Prenatal Testing for SHH, ZIC2, SIX3, and TGIF Gene Variants: Holoprosencephaly

Panel Gene List: SHH, ZIC2, SIX3, TGIF

Clinical Features:
Clinical features in Newborns and Children: Holoprosencephaly (HPE) is a developmental anomaly of the forebrain and midface. There is a wide spectrum of severity, ranging from cyclopia with proboscis above the single eye as the most severe/complete presentation, to ocular hypotelorism and a single central upper incisor at the mild end of the spectrum (microform). About 80% of individuals with HPE have some characteristic associated facial anomalies. Developmental delay is seen in all individuals with HPE-associated brain anomaly.

Prenatal Ultrasound Findings: SHH, ZIC2, SIX3, and TGIF genetic testing should be considered in fetuses with evidence of holoprosencephaly. Alobar holoprosencephaly is typically identified on prenatal ultrasound after 16 weeks' gestation;[1] however, milder forms of HPE, including semilobar and lobar holoprosencephaly, may not be detectable. In some cases, if prenatal ultrasound identifies a brain anomaly suggestive of possible HPE, fetal MRI may be useful to help clarify the diagnosis. In addition to HPE, prenatal imaging studies may also detect a cleft lip or hydrocephalus in some cases. Ultrasound examination may be normal in affected fetuses; therefore, pregnancies at risk to inherit a specific known familial pathogenic variant can be offered targeted molecular testing regardless of ultrasound findings, if desired.

Inheritance Pattern/Genetics:
HPE is etiologically heterogeneous, with genetic factors, maternal host factors, and possibly environmental factors contributing. In families with an identified variant in one of the known HPE genes, most variants occurred de novo, although autosomal dominant inheritance with greatly reduced penetrance in the segregating parent is not uncommon. Rarely, HPE families with a complex, recessive inheritance have been reported, with the proband having a variant in each of two different HPE-associated genes and each parent being a clinically normal carrier of one of the variants.

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 a deletion or duplication of one or more exons of these genes. For known familial variants, the relevant portion of the appropriate gene will be analyzed in duplicate.

Additionally, genotype analysis of maternal and fetal DNA for several polymorphic markers to test for maternal cell contamination will be performed. Therefore, in all prenatal cases a maternal sample should accompany the fetal sample.

Test Sensitivity:

Approximately 18-25% individuals with holoprosencephaly have 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% will have a variant in the SHH, TGIF, SIX3, or ZIC2 gene identifiable by DNA sequencing. Such variants are usually are 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 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.[2] Additionally, microdeletions were identified in 8 of 94 (8.5%) fetuses with a normal karyotype and no point variants.[3]

The phenotype associated with variants in the nonsyndromic HPE genes is extremely variable. Patients with ZIC2 variants may have milder facial characteristics, despite severe CNS involvement. 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:

1. Blaas et al., (2000) Obstet Gynecol 15:62-65.
2. Bendavid et al. (2006) J Med Genet 43: 496-500.
3. Bendavid et al. (2006) Hum Genet 119:1-8.
4. Wallis et al. (2000) Human Mutat 16:99-108.
5. Muenke, M and Gropman, A. Holoprosencephaly Overview. In: GeneClinics: Clinical Genetic Information Resource [database online]. Copyright, University of Washington, Seattle. Available at http://www.genetests.org. (Mar 2008).