Prenatal genetic testing of TSC1 and TSC2 Genes in Tuberous Sclerosis Complex

Clinical Features:

Tuberous sclerosis complex (TSC) is characterized by abnormalities of the skin, brain, kidney, heart, and lungs. Skin findings are present in nearly all patients with TSC, and major criteria in skin include facial angiofibromas, forehead plaque, nontraumatic ungual or periungual fibromas, three or more hypomelanotic macules, or a shagreen patch. Major features involving other body systems include multiple retinal nodular hamartomas, cortical tuber, subependymal nodule, subependymal giant cell astrocytoma, cardiac rhabdomyoma, lymphangiomyomatosis, and renal angiomyolipoma. Minor features include randomly distributed pits in dental enamel, hamartomatous rectal polyps, bone cysts, cerebral white matter radial migration lines, gingival fibromas, non-renal hamartoma, retinal achromic patch, confetti skin lesions, and multiple renal cysts. Individuals who meet diagnostic criteria for definite TSC have two major features or one major and two minor features, probable TSC requires one major plus one minor feature, and possible TSC is one major or two or more minor features.[2]

In addition to the clinical diagnostic criteria listed above, individuals with TSC have a significantly increased risk for other neurodevelopmental disorders. Approximately 50% have intellectual disability or developmental delay and 40% have autism spectrum disorders. Additionally, greater than 80% have seizures, including infantile spasms with hypsarrhythmia.

Rarely, individuals with TSC may also exhibit features of polycystic kidney disease (PKD), which results in multiple renal cysts often leading to end-stage renal disease and also increases the risk for Berry aneurysms and for cysts in other organs. Individuals with features of both TSC and PKD typically have a contiguous gene deletion syndrome involving the TSC2 and PKD1 genes.

The major features of TSC which may be detected prenatally include: cardiac rhabdomyomas, cortical tubers, subependymal nodules, and renal angiomyolipomas.[3] Cardiac rhabdomyomas are typically identified as incidental findings on ultrasound in the second trimester.[4] Cortical tubers can be detected as early as 20 weeks gestation.[5,6] The absence of brain and/or renal lesions on prenatal ultrasound or MRI in a fetus with cardiac rhabdomyomas does not exclude a diagnosis of TSC, as these lesions may not be detectable or may not develop until after birth. In a 2009 prospective study of 51 fetuses prenatally diagnosed with cardiac rhabdomyomas, Saada et al. found 10 cases with a single cardiac rhabdomyoma and 41 with multiple cardiac rhabdomyomas. Cerebral MRI was performed between 24 and 37 weeks gestation and found 25 fetuses with a brain lesion specific to TSC, leading to a prenatal clinical diagnosis in 25/51 (49%) fetuses. Of the 26 fetuses with normal MRI, follow up evaluation led to clinical diagnosis

of TSC in an additional 14 cases (3 at autopsy, 6 on postnatal MRI, and 4 due to characteristic skin lesions after birth). Therefore, in this study, 39 of the 51 (76%) fetuses with prenatally diagnosed cardiac rhabdomyomas were found to have a clinical diagnosis of TSC.

Inheritance Pattern/Genetics:
TSC is inherited in an autosomal dominant manner. Approximately 1/3 of cases are familial and 2/3 of cases have de novo variants. Somatic mosaicism has been described and is estimated to be present in approximately 1% of patients with TSC.[9,1] Germline mosaicism has also been reported in numerous families with TSC, and the recurrence risk for siblings of a proband with an apparent de novo variant is estimated to be 1-3%.[8]

Test Methods:
Using genomic DNA extracted from the submitted specimen, the complete coding regions and splice site junctions of the genes tested 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; however, technical limitations and inherent sequence properties effectively reduce this resolution for some genes. Alternative sequencing or copy number detection methods are used to analyze or confirm regions with inadequate sequence or copy number data by NGS. 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. Deletions/duplications including the 3' end of the TSC2 gene (exons 36-42) may not be detected by this testing.

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:
Overall, approximately 80-85% of individuals who meet clinical diagnostic criteria for TSC have a detectable pathogenic variant in either the TSC1 or TSC2 gene.[12-15] Specifically, 15-17% of

individuals have pathogenic variants in theTSC1 gene while 50-65% have pathogenic variants in the TSC2 gene, and the remaining do not have an identifiable genetic cause for their features.[12,14,15] In one report, 17 of 23 cases of prenatally diagnosed cardiac rhabdomyoma were available for a clinical workup of TSC following birth. Of the 17 cases, 9 individuals met the clinical criteria for a diagnosis of TSC . Molecular genetic testing was completed on 4 of the 9 patients and all four individuals were found to harbor a pathogenic variant in either the TSC1 or TSC2 gene.[16]

References:

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