Genetic testing of TSC1 and TSC2 Genes in Tuberous Sclerosis Complex (TSC)

Mendelian Inheritance in Man Number: 191100 (tuberous sclerosis 1); 613254 (tuberous sclerosis 2); 605284 (TSC1 gene); 19102 (TSC2 gene)

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, lymphangiomatomatosis, 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 achromatopsic 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 (Roach and Sparagano 2004).

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. Nearly ¾ of individuals with TSC who have infantile spasms respond to treatment with vigabatrin (Camposano et al., 2008; Northrup et al., 2015).

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.

Inheritance pattern:
TSC is inherited in an autosomal dominant manner. Approximately 1/3 of cases are familial and 2/3 are de novo. Somatic mosaicism has been described and is estimated to be present in up to 7-8% of patients with TSC (Tyburczy et al., 2015; Qin et al., 2010; Northrup et al., 2015). 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% (Rose et al., 1999; Northrup et al., 2015).

Reasons for referral:
1. Confirmation of a clinical diagnosis
2. Carrier testing for individuals with a known familial TSC1 or TSC2 variant
3. Prenatal diagnosis in at-risk pregnancies

Genes/Proteins:
Both TSC1 and TSC2 are tumor suppressor genes that together regulate the mTOR pathway, which has a critical role in controlling cell size and proliferation (Northrup et al., 2015). The TSC1 gene encodes the hamartin protein, which is involved in regulation of the cell-cycle, neurite growth, synapse formation, and axon development. The TSC2 gene encodes the tuberin protein, which plays a role in protein translation as well as cell growth and proliferation. Additionally, hamartin and tuberin interact to facilitate GTPase activation.

Variant Spectrum:
Approximately 80% of TSC1 variants and 65% of TSC2 variants are nonsense, splice site, or frameshift variants, while missense substitutions account for 17% of TSC1 variants and 26% of TSC2 variants. (Mayer et
Large deletions or duplications encompassing one or more exons account for ~0.5% of TSC1 variants and 6% of TSC2 variants (Kozlowski et al., 2007).

Genotype/Phenotype Correlation:
Pathogenic variants in TSC1 and TSC2 cause overlapping clinical phenotypes, although in general TSC2 variants are associated with a more severe clinical presentation (Northrup et al., 2015). Individuals with TSC2 variants have been reported to have a higher likelihood of developing renal malignancy, intellectual disability, infantile spasms, and autism spectrum disorders than individuals with TSC1 variants.

Test method:
Using genomic DNA, coding exons and flanking splice junctions of the genes on this panel are enriched using a proprietary targeted capture method developed by GeneDx. The products are sequenced on an Illumina instrument using paired end reads. The sequence data is aligned to reference sequences based on human genome build GRCh37/UCSC hg19. Sanger sequencing is used to compensate for low coverage and refractory amplifications. Concurrently, targeted array CGH analysis with exon-level resolution is performed to evaluate for a deletion or duplication of one or more exons of the genes included on the panel. Deletions/duplications including the 3' end of the TSC2 gene (exons 36-42) may not be detected by this testing. The presence of any potentially disease-associated sequence variant(s) or copy number alteration(s) is confirmed by dideoxy DNA sequence analysis or quantitative PCR, respectively, or by other appropriate methods. If clinically appropriate, if the TSC panel is negative, sequencing and deletion/duplication analysis of the remaining 85 genes on the Comprehensive Epilepsy Panel is available as a separate test.

Test sensitivity:
Overall, approximately 80-85% of individuals who meet clinical diagnostic criteria for TSC have a detectable variant in the TSC1 or TSC2 genes (Jones et al., 1999; Dabora et al., 2001; Sancak et al., 2005; Au et al., 2007). Specifically, 15-17% of individuals have TSC1 variants while 50-65% have TSC2 variants, and the remaining do not have an identifiable genetic cause for their features (Jones et al., 1999; Sancak et al., 2005; Au et al., 2007). The technical sensitivity of this sequencing test is estimated to be 98%. It will not reliably detect deletions, insertions, or rearrangements greater than or equal to ten base pairs. The deletion/duplication testing can detect deletions or duplications encompassing one or more exons, including variants as small as 150-300 bp.

Specimen Requirements and Shipping/Handling:
- **Blood:** 2-5 ml whole blood in EDTA (lavender-top). 1-2 ml is acceptable from infants. Ship blood overnight at ambient temperature, using a cool pack in hot weather. Blood specimens may be refrigerated for up to 7 days prior to shipping.
- **Extracted DNA:** Outside DNA is discouraged; however, high quality extracted DNA can be accepted. Follow instructions at [http://www.genedx.com/order-a-test/specimen-requirements/](http://www.genedx.com/order-a-test/specimen-requirements/)
- **Oral Rinse:** Use GeneDx kit only. Follow instructions at [http://www.genedx.com/order-a-test/specimen-requirements/](http://www.genedx.com/order-a-test/specimen-requirements/)
- **Other specimens:** Contact us for specific information.
- **Prenatal Diagnosis (for specific known familial mutation(s) or deletion(s) only):** For prenatal testing for a known pathogenic variant in an epilepsy gene, please refer to the specimen requirements table on our website at: [http://www.genedx.com/test-catalog/prenatal/](http://www.genedx.com/test-catalog/prenatal/). Ship specimen overnight at ambient temperature, using a cool pack in hot weather.

For test codes, CPT codes, and turn-around-times, please refer to the TSC1 and TSC2 testing page on our website: [www.genedx.com](http://www.genedx.com)

References Cited: