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

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. Nearly ¾ of individuals with TSC who have infantile spasms respond to treatment with vigabatrin[1,12].

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.

Pathogenic variants in TSC1 and TSC2 cause overlapping clinical phenotypes, although in general TSC2 variants are associated with a more severe clinical presentation. [1] 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.

INHERITANCE PATTERN/GENETICS

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[1,4,11]. 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%[1,3].

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. [1] 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.

TEST METHODS

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.

CLINICAL SENSITIVITY

Overall, approximately 80-85% of individuals who meet clinical diagnostic criteria for TSC have a detectable variant in the TSC1 or TSC2 genes[7,8,9,10]. 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[7,8,10]. 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.

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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.[5] Large deletions or duplications encompassing one or more exons account for ~0.5% of TSC1 variants and 6% of TSC2 variants. [6]

REFERENCES:

1. Northrup H, Koenig MK, Au KS. Tuberous Sclerosis Complex. 1999 Jul 13 [Updated 2015 Sept 3]. In: Pagon RA, Adam MP, Bird TD, et al., editors. GeneReviews™ [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2013. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1220/.
2. Roach and Sparagano (2004) J Child Neurol 19:643-649.
3. Rose et al., (1999) Am J Hum Genet 64:986-992.
4. Qin et al., (2010) Hum Genet 127:573-582.
5. Mayer et al., (2013) Eur J Hum Genet e1–e4; doi:10.1038/ejhg.2013.129.
6. Kozlowski et al., (2007) Hum Genet 121:389-400.
7. Jones et al., (1999) Am J Hum Genet 64:1305-1315.
8. Dabora et al., (2001) Am J Hum Genet 68:64-80.
9. Sancak et al., (2005) Eur J Hum Genet 13:731-741.
10. Au et al., (2007) Genet Med 9:88-100.
11. Tyburczy et al., (2015) PLoS Genet 11(11): e1005637. doi: 10.1371/journal.pgen.1005637.
12. Camposano et al., (2008) Epilepsia 49:1186-1191.