

Assay Summary

PTEN Gene Mutation Analyses PTEN hamartoma tumor syndrome (PHTS)

Synopsis

The germline mutations in the *PTEN* gene are associated with a collection of phenotypically distinct hamartomatous overgrowth syndromes including Cowden syndrome (CS), Bannayan-Riley-Ruvalcaba syndrome (BRRS), Proteus syndrome (PS), and Proteus-like syndrome^{1,2}. The *PTEN* is a tumor suppressor gene located at [10q23.3](#) and encodes a lipid phosphatase which signals down the phosphoinositol-3-kinase/Akt pathway and leads to G1 cell cycle arrest and apoptosis^{3,4}. The germline mutations in *PTEN* gene are associated with about 85% of Cowden syndrome (CS), 65% of Bannayan-Riley-Ruvalcaba syndrome (BRRS), up to 20% of Proteus syndrome (PS), and approximately 50% of a Proteus-like syndrome (PSL) cases^{4,5,6}. *PTEN* mutations can also be associated with Autism Spectrum Disorders (ASD)⁹. The mutations for *PTEN* hamartoma tumor syndrome are inherited in an autosomal dominant manner².

Cowden syndrome (CS) – The Cowden Syndrome is a multiple hamartoma cancer predisposition syndrome associated with an increased risk of malignant and benign tumors of breast, thyroid, and endometrial cancers. It also characterized by hamartomas, mucocutaneous lesions (facial trichilemmomas, papillomatous papules, acral and plantar keratosis), adult Lhermitte-Duclos disease (LDD), autism, mental retardation (12%) and macrocephaly as well as benign manifestations^{3,8}. The estimated prevalence of CS is 1 in 200,000 individuals, though that is believed to be an under estimation⁴. Almost 40-60% *PTEN* mutations are inherited in CS and others are denovo⁴.

The lifetime risk for individuals with CS is estimated to be 25-50% for breast cancer (for women), about 10% for thyroid cancer (for both sexes) and 5-10% for endometrial cancer (for women)⁷.

Bannayan-Riley-Ruvalcaba syndrome (BRRS) – It is a congenital disorder that is characterized by macrocephaly, lipomatosis, hemangiomas, intestinal polyposis, pigmented macules of the penis, macrosomia, proximal muscle myopathy, joint hyperextensibility, pectus excavatum, scoliosis, developmental delay or mental retardation¹.

Proteus syndrome (PS): It is an extremely rare and variable disorder characterized by progressive, asymmetric, and disproportionate overgrowth. Some of the key features are malformations and hamartomatous overgrowths of tissues, connective tissue nevi, epidermal nevi, parotid adenomas, vascular malformations, hyperostosis, lung cysts, facial abnormalities, hamartomatous tissue overgrowth, hyperostoses, connective tissue and epidermal nevi, dysregulated adipose tissue, vascular malformations and other congenital malformations¹.

Proteus-like syndrome (PSL): The individuals with some of the characteristics of Proteus syndrome but who do not meet clinical diagnostic criteria for it are diagnosed with Proteus-like syndrome¹.

Autism Spectrum Disorders (ASD) – Autism spectrum disorders are characterized by impaired social relationships, impaired language and communication, and a narrow range of interests or repetitive behaviour¹⁰. *PTEN* gene plays an important role in brain development and plasticity. Although the exact frequency of *PTEN*

mutations in patients with ASD is uncertain, they may be found in up to 17 % patients with autism and macrocephaly⁹.

Indications for testing

- 1) Individuals that are suspected as having one of the PTEN hamartoma tumor syndromes.
- 2) Individuals with a family history of a PTEN hamartoma tumor syndrome.
- 3) Individuals with a family history of PTEN mutation.

Methodology

PTEN sequence analysis: All coding exons (exons 1-9), associated intron junctions of the PTEN gene, and the promoter region are analyzed by direct DNA sequence analysis using an automated fluorescent sequencing machine. When a mutation is detected, confirmation is carried out on an independent amplification of PCR using a second prep (B-prep) by sequencing in the opposite direction. If no mutation is found, sequence analysis is performed in both directions. At-risk family members can be offered DNA sequence analysis of only the region of the gene with the previously identified mutation.

PTEN MLPA analysis: We have incorporated the SALSA MLPA (multiplex ligation-dependent probe amplification) kit that is a rapid, high throughput technique for copy number quantification, specifically testing for large deletions/duplications for the exons 1-9 of PTEN gene. This assay should be considered for patients where full gene sequencing did not detect a mutation in the PTEN gene.

Limitations

For mutation analysis, the method will not detect mutations located in regions of the genes that are not analyzed (non-coding exon regions, intron regions other than the splice junctions, and upstream and downstream regions). Some sequence alterations that may be detected (such as those causing missense or synonymous changes) will be of unknown clinical significance. Interpretation of test results should be in the context of the patient's ethnicity, clinical and family histories, and other laboratory test results.

Specimen Requirements

- (a) Blood samples: 2 tubes with a total of 6 ccs in ACD (yellow top) or EDTA (lavender top) tubes. Keep at ambient temperature and ship by overnight courier. Samples must be received in our laboratory within 72 hours of draw.

Note:

- i) for infants, a minimum of 3 ccs is sufficient.
- ii) we accept DNA; at least 10 micrograms is required.

- (b) Prenatal samples: 2 T25 flasks of confluent cells sent padded to arrive on M/Tu/W. A blood sample from the mother maybe required (2 tubes with a total of 6 ccs in ACD (yellow top) or EDTA (lavender top) tubes) for use as positive control. Maternal cell contamination studies are not done here but are required for autosomal disorders and dosage analysis on X-linked disorders. We would be happy to assist in coordinating sending out a specimen for this purpose.

Test Request Form (TRF)

- a) A completed MDL [TRF](#) is required for each specimen. Please submit the completed TRF with the specimen. Complete testing and billing information must be provided before the specimen is processed.
- b) [PTEN Patient Information Form](#): Include a completed PTEN Patient Information .

<i>Order Codes</i>	<i>CPT Codes</i>	<i>TAT</i>
PTEN-SEQ (PTEN gene, full gene sequencing)	83890, 83898(x10), 83894, 83904(x10), 83912	4 wks
PTEN-CAS (PTEN gene, targeted mutation analysis, known mutation)	83890, 83898, 83894, 83904, 83912	3 wks
PTEN-PD (PTEN gene, known mutation detection, prenatal)	83890, 83898, 83894, 83904, 83912	2 wks
PTEN-DEL (PTEN gene, MLPA analysis)	83890, 83896(x25), 83909, 83912	3 wks
PTEN-DEL-CAS (PTEN gene, MLPA analysis, known deletions/duplications)	83890, 83896(x25), 83909, 83912	3 wks
PTEN-DEL-PD (PTEN gene, MLPA analysis, prenatal)	83890, 83896(x25), 83909, 83912	3 wks

References

1. Blumenthal, G.B. and Dennis, P.A. (1998). *European Journal of Human Genetics* 16, 1289–1300.
2. Orloff, M.S. and Eng, C. (2008). *Oncogene* 27:5387–5397.
3. Eng, C. (2003). *Human Mutation* 22:183-198.
4. Zhou, X.P. et al. (2003). *Am. J. Hum. Genet.* 73:404–411.
5. Maehama, T. et. al. (2004). *Biochemical Society Transactions* 32;332-347.
6. Teresi, R.E. et al. (2007). *Am. J. Hum. Genet.*81:756–767.
7. Zbuk, K.M. and Eng, C. (2007). *Gastroenterology and Hepatology* 4:492-502.
8. Hanssen A.M.N. and Fryns, J.P. (1995). *J Med Genet.* 32:117–119.
9. Butler M.G., et.al. (2005). *J Med Genet.* 42:318–21
10. Pardo, C.A. and Eberhart, C.G. 2007 *Brain Pathol.* 17:434–447.

NOTE: This test is performed pursuant to a license agreement with Roche Molecular Systems, Inc.