City of Hope

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Assay Summary

PTEN Gene Mutation Analyses
PTEN hamartoma tumor syndromes (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 manner[2].

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 estimation[4]. Almost 40-60% PTEN mutations are inherited in CS and others are denovo[4].
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 retardation[1].

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 malformations[1].

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 syndrome[1].

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 behaviour[10]. 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 % patient s with autism and macrocephaly [9].

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: Coding exons and associated intron junctions are captured and enriched using custom Agilent SureSelect technology. Next-generation sequencing is performed on Illumina MiSeq. Additional Sanger sequencing is performed for any regions with insufficient depth of coverage or for verification of suspect variant calls. Targeted testing for known familial mutation is performed by Sanger sequencing.

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

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.

Test Request Form (TRF)

a) A completed CMDL 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 .

Order Codes	*CPT Codes*	*TAT*
PTEN-SEQ (PTEN gene, full gene sequencing by NGS)	81321, G0452	3 wks
PTEN-CAS (PTEN gene, targeted mutation analysis, known mutation)	81322, G0452	2 wks
PTEN-DEL (PTEN gene, MLPA analysis)	81323, G0452	3 wks
PTEN-DEL-CAS (PTEN gene, MLPA analysis, known deletions/duplications)	81323, G0452	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.