City of Hope

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Assay Summary

Germline *EPCAM* (epithelial cell adhesion molecule, *TACSTD1*) Gene Deletion MLPA analysis

Synopsis

Hereditary nonpolyposis colorectal cancer (HNPCC), also known as Lynch syndrome patients are at an increased risk for colorectal and several other cancers owing to inactivating germline mutations in mismatch repair genes (MMR), including *MLH1*, *MSH2*, *MSH6* and *PMS2*. Recently, several groups reported that germline *EPCAM* deletions in 3' region can cause HNPCC. The *EPCAM* deletions result in loss of the exons 8 and 9 (the last two exons), including the polyadenylation signal, which abolished transcription termination. This leads to transcription read-through into the downstream of *MSH2* gene causing methylation of the *MSH2* promoter and ultimately silencing of *MSH2* gene[1].

Kovacs M. E. et al reported that the *EPCAM* deletion was found in about 19% HNPCC families without identifiable pathogenic variant in MMR genes[3]. Niessen R.C. et al found that the overall contribution of *EPCAM* deletion to the HNPCC cases is about 6.3% [2]. The mean age of diagnosis in the patients is 43 years, which is comparable with the average age of onset in patients with HNPCC with a germline MMR gene mutation [1, 2]. Although more clinical data will be needed to set up criteria of the clinical managements, it seems reasonable to provide *EPCAM* deletion patients the standard HNPCC surveillance protocols.

Indications for testing

1) Patients that meet any of the criteria for HNPCC but negative for germline mutations in MLH1, MSH2, MSH6 and PMS2.
2) Patients with MSH2 absent on immunohistochemistry (IHC) but without germline mutations in MSH2.

Methodology

We have incorporated the SALSA Multiplex Ligation-Dependent Probe Amplification (MLPA) kit which is a rapid, high-throughput technique for copy number quantification, specifically testing for large deletions for the *EPCAM* gene in HNPCC. The P072 kit contains probes for exons 3, 8 and 9 of the *EPCAM* and several probes covered 2.5 kb upstream of *MSH2* and 3kb downstream of *EPCAM*. The MLPA products are analyzed by DNA fragment analysis on an automated fluorescent sequencer. The absence or presence of deletions of one or more exons is confirmed by MLPA analysis using an independently amplified segment. Heterozygote deletions of probe recognition sequences should give a 35-50% reduced relative peak area of the amplification product of that probe.

Performance

Large *EPCAM* deletions are estimated to account for approximately 6-19% of HNPCC patients [2, 3]. We have incorporated the SALSA MLPA kit which is a rapid, high-throughput technique for copy number quantification, specifically testing for large deletions for the gene. The assay sensitivity of deletion detection is >99%.

Limitations

This SALSA MLPA kit is designed to detect deletions of one or more exons of the *EPCAM* gene. Heterozygous deletions of probe recognition sequences should give a 35-50% reduced relative peak area of the amplification product of that probe. Mutations and/or polymorphisms very close to the probe ligation site may also result in a reduced relative peak area. Therefore, apparent deletions detected by a single probe always require confirmation

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by other methods. MLPA analysis will not detect sequence alterations or inversions. 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) HNPCC Patient Information Form: Include a completed HNPCC Patient Information .

Order Codes	***CPT Codes***	***TAT***
EPCAM-DEL (EPCAM gene, MLPA analysis (Exons 3, 8, 9))	81403, G0452	3 wks
EPCAM-DEL-CAS (EPCAM gene, MLPA analysis, known deletions/duplications (Exons 3, 8, 9))	81403, G0452	3 wks

References

1. Ligtenberg M. J. et al., (2009) Nat Genet. 41(1):112-7
2. Niessen R.C. et al., (2009) Genes Chromosomes Cancer; 48(8): 737-44
3. Kovacs M. E. et al., (2009) Hum Mutat. 30(2):197-203

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