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Assay Summary

Hereditary Nonpolyposis Colorectal Cancer

MLH1, MSH2, MSH6, and PMS2 Gene Mutation Analyses

Synopsis

Inherited (germline) mutations in several genes involved in DNA mismatch repair are the major cause of hereditary nonpolyposis colorectal cancer (HNPCC), also known as Lynch syndrome. The syndrome predominantly shows autosomal dominant inheritance (only one copy of a mutation is necessary for expression of disease) and incomplete penetrance (not all mutation carriers will get the disease). However, there have been reports of early onset cases with biallelic mutations in MSH6 and PMS2.

HNPCC comprises about 5-10% of all colon cancer and confers up to an 80% lifetime risk of developing colon cancer, a 35% risk for endometrial cancer, a 9% risk for ovarian cancer, and a small risk (<10%) for several other extracolonic cancers as well¹. At least five human genes (MLH1^{1,2}, MSH2^{3,4}, MSH6^{5,8-13}, PMS1⁶, PMS2⁶) have been implicated in HNPCC. Alterations of the MLH1 and MSH2 genes account for a majority of mutations in HNPCC. Alterations of the MSH6 are estimated to account for approximately 7-10% of mutations in HNPCC^{5,12,14}. It has been suggested that germline MSH6 mutations predispose a special subset of HNPCC individuals to primarily late-onset, familial colorectal or endometrial carcinomas that often do not fulfill classic criteria for HNPCC^{9,12,13}. MSH6 mutations have also been detected in patients with tumors that show no or low microsatellite instability.^{10,11} The prevalence of PMS2 mutations is not as well established, though one paper reported a 4% detection rate in 97 Lynch syndrome cases that were negative for mutations in MLH1, MSH2, and MSH6¹⁹. The detection rate jumps to approximately 60% if the patient is found to have abnormal staining for PMS2 by immunohistochemistry²⁰. Interestingly, a number of biallelic PMS2 cases have been reported. One recent report described an individual with childhood onset malignancy and café-au-lait spots who was found to be biallelic for PMS2 mutations²⁰. Other studies have reported similar phenotypes in biallelic cases with MSH6 or PMS2 mutations^{21,22}. Common features include CNS tumors, leukemia, and café-au-lait spots. Large deletions or genetic rearrangements account for 5-10% of mutations in *MLH1* and 17-50% of mutations in *MSH2* gene²⁴. Large deletions of the MSH6 gene are estimated to account for approximately 2-3% of mutations in this gene¹⁸.

Alterations to these genes are predominantly single base pair changes, or small insertions/deletions. However, larger single and multi-exon deletions have also been reported. Sequencing based analysis is able to detect the latter, but the larger heterozygous deletions require some sort of quantitative analysis (see MLPA analysis below).

Indications for testing

Individuals with cancer that meet the revised guidelines established by a National Cancer Institute Workshop on Hereditary Nonpolyposis Colorectal Cancer Syndrome ("Revised Bethesda Guidelines")⁷ are candidates for MSI testing. Those individuals meeting Bethesda Guidelines whose tumors show high microsatellite instability are very likely to carry germline mutations in one of the mismatch repair genes. Additionally, individuals with abnormal immunohistochemistry (IHC) results are very likely to carry germline mutations in one of the mismatch repair genes. The IHC results itself can provide guidance as to

which specific gene/s to test. This test should be offered in the context of genetic counseling before and after testing.

Methodology

MLH1, MSH2, MSH6, and PMS2 sequence analysis: Full Mutation Analysis of MLH1, MSH2, MSH6, and PMS2: All coding exons and associated intron junctions of the MLH1 gene, the MSH2 gen, the MSH6 gene, and/or the PMS2 gene are analyzed by direct DNA sequence analysis using an automated fluorescent sequencer. 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 the upstream and downstream directions. At-risk family members can be offered DNA sequence analysis of only the region of the gene with the previously identified mutation.

MLH1, MSH2 and MSH6 MLPA analysis for large deletions or genetic rearrangements: 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 MLH1, MSH2 and MSH6 gene in HNPCC. The P003 contains probes for each of the 19 exons of the MLH1 gene, for each of the 16 exons of the MSH2 gene; the P072 MSH6 probemix contains probes for each of the 10 MSH6 exons. The MLPA assay was optimized, and validated to screen for large deletions.

Performance

MLH1, MSH2, MSH6 and PMS2 sequence analysis: MLH1 and MSH2 account for approximately 90% of families with HNPCC¹⁴. MSH6 accounts for approximately 7% of families with HNPCC^{5, 12, 14}, and PMS2 mutations likely account for slightly less. If a point mutation or small deletion/insertion exists within the regions of the MLH1, MSH2, MSH6, or PMS2 genes that are sequenced, the sensitivity of mutation detection is approximately 99% (Analytical sensitivity).

MLH1, MSH2 and MSH6 MLPA large deletion analysis: Large deletions are estimated to account for approximately 5-10% of MLH1 mutation²⁴, 17-50% of MSH2 mutations^{15, 16, 17} and 2-3% of MSH6¹⁸. We have incorporated the SALSA MLPA kit which is a rapid, high-throughput technique for copy number quantification, specifically testing for large deletions/duplications for the gene. This assay should be considered for patients with HNPCC where full gene sequencing did not detect a mutation. The assay sensitivity of deletion detection is >99%.

Limitations

The mutation analysis 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). The method also will not detect gross genetic alterations including most duplications, inversions, or deletions. Some sequence alterations that may be detected (such as those causing missense or synonymous changes) will be of unknown clinical significance. Additionally, there are a number of established PMS2 pseudogenes. The PMS2 assay was designed with primers to selectively amplify the PMS2 gene, while avoiding these pseudogenes. However, gene conversion has been reported to occur, which could interfere with the interpretation of certain alterations. As HNPCC is genetically heterogeneous, mutations in genes other than MLH1, MSH2, MSH6, and PMS2 are possible; these other genes are not analyzed by this assay.

This SALSA MLPA kit is designed to detect deletions / duplications of one or more exons of the HNPCC genes. Heterozygous deletions of probe recognition sequences should give a 35-50% reduced relative peak area of the amplification product of that probe. However, 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 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

- (a) Blood samples: 2 tubes (6 ccs each) in ACD (yellow top) or EDTA (lavender top) tubes.
Keep 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 6 ccs is sufficient
- ii) we accept DNA; at least 10 micrograms

- (b) Prenatal samples: 2 T25 flasks of confluent cells sent padded to arrive on M/Tu/W/Th.
A blood sample from the mother is also required (6 ccs in ACD, yellow top) 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) [HNPCC Patient Information Form](#): Include a completed HNPCC Patient Information .

| Order Codes | CPT Codes | TAT |
|---|---|-------|
| MLH1-SEQ (MLH1 gene, full gene sequencing) | 83890, 83898(x20), 83904(x20), 83894, 83912 | 6 wks |
| MLH1-CAS (MLH1 gene, targeted mutationa analysis) | 83890, 83898, 83904, 83894, 83912 | 4 wks |
| MLH1-DEL (MLH1 gene, MLPA analysis) | 83890, 83896(19), 83909, 83912 | 2 wks |
| MLH1-DEL-CAS (MLH1 gene, MLPA analysis, known mutation) | 83890, 83896(19), 83909, 83912 | 2 wks |
| MLH1-DEL-PD (MLH1 gene, MLPA analysis, prenatal) | 83890, 83896(19), 83909, 83912 | 2 wks |
| MSH2-SEQ (MSH2 gene, full gene sequencing) | 83890, 83898(x16), 83904(x16), 83894, 83912 | 6 wks |
| MSH2-CAS (MSH2 gene, targeted mutationa analysis) | 83890, 83898, 83904, 83894, 83912 | 4 wks |
| MSH2-DEL (MSH2 gene, MLPA analysis) | 83890, 83896(18), 83909, 83912 | 3 wks |
| MSH2-DEL-CAS (MSH2 gene, MLPA analysis, known mutation) | 83890, 83896(18), 83909, 83912 | 3 wks |
| MSH2-DEL-PD (MSH2 gene, MLPA analysis, prenatal) | 83890, 83896(18), 83909, 83912 | 3 wks |
| MSH6-SEQ (MSH6 gene, full gene sequencing) | 83890, 83898(x11), 83904(x19), 83894, 83912 | 6 wks |
| MSH6-CAS (MSH6 gene, targeted mutationa analysis) | 83890, 83898, 83904, 83894, 83912 | 4 wks |
| MSH6-DEL (MSH6 gene, MLPA analysis) | 83890, 83896(15), 83909, 83912 | 3 wks |
| MSH6-DEL-CAS (MSH6 gene, MLPA analysis, known mutation) | 83890, 83896(15), 83909, 83912 | 3 wks |
| MSH6-DEL-PD (MSH6 gene, MLPA analysis, prenatal) | 83890, 83896(15), 83909, 83912 | 3 wks |
| PMS2-SEQ (PMS2 gene, full gene sequencing) | 83890, 83898 (x16), 83904 (x15), 83894, 83912 | 8 wks |
| PMS2-CAS (PMS2 gene, targeted mutationa analysis) | 83890, 83898, 83904, 83894, 83912 | 4 wks |

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NOTE: This test is performed pursuant to a license agreement with Roche Molecular Systems, Inc. MSH2 analysis is licensed under U.S. Patent Nos. 5,693,470 and 5,837,443.