City of Hope

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Assay Summary

Lynch Syndrome/HNPCC Testing

MLH1, MSH2, MSH6, PMS2 and EPCAM Genes Mutation Analysis

Synopsis

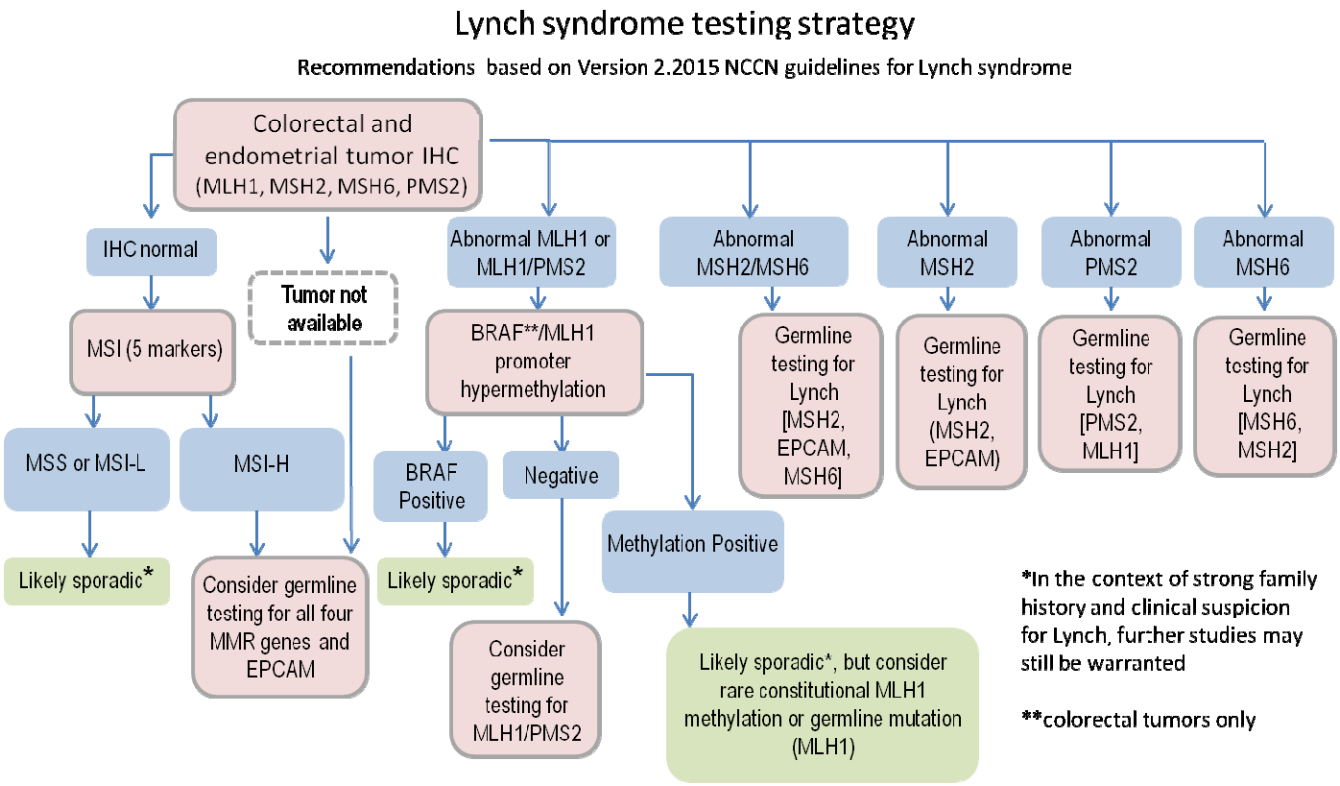
Lynch syndrome, also known as hereditary nonpolyposis colorectal cancer or HNPCC, is a cancer predisposition syndrome caused by heritable mutations in specific genes involved in DNA mismatch repair (MMR). It accounts for about 5% of all colon cancer and confers up to an 80% lifetime risk of developing colon cancer, up to 60% risk for endometrial cancer, up to 12% risk for ovarian cancer, and a lower risk for several other extracolonic cancers[1]. Four MMR genes, *MLH1*, *MSH2*, *MSH6*, *PMS2*, have been implicated in Lynch syndrome.

MLH1 and *MSH2* genes account for the majority of pathogenic mutations in Lynch syndrome. Mutations in *MSH6* are estimated to account for approximately 7-10% and have been more often associated with late-onset, familial colorectal or endometrial carcinomas that often do not fulfill classic criteria for Lynch[2-4]. *MSH6* mutations have also been detected in patients with tumors that show no or low microsatellite instability[5]. The prevalence of *PMS2* mutations is not as well established, although a 4% detection rate has been reported in 97 Lynch syndrome cases negative for mutations in *MLH1*, *MSH2*, and *MSH6*[6]. The likelihood of *PMS2* mutation increases significantly for patients with isolated absence of PMS2 observed on tumor immunohistochemistry[7]. Lynch syndrome is generally inherited in an autosomal dominant manner with incomplete penetrance; however biallelic mutations of MMR genes have also been reported and associated constitutional mismatch repair deficiency (CMMR-D), characterized by a spectrum of earlier onset malignancies and features such as CNS tumors, leukemia, and café-au-lait spots[7-10].

Large deletions or genetic rearrangements are estimated to account for ~10% of mutations in *MLH1*, 17-50% of *MSH2*, 2-3% of *MSH6*, and 21-37 % of *PMS2* gene mutations[11-13]. A unique class of Lynch syndrome mutation is deletion including the 3' end and the last exons of the *EPCAM* gene which results in hypermethylation and transcriptional silencing of the adjacent *MSH2* gene[14,15].

Indications for testing

Germline testing for MMR gene mutations is indicated as part of a the testing strategy for individuals with Lynch syndrome or suspected diagnosis of Lynch syndrome. Individuals meeting Bethesda or Amsterdam criteria whose tumors show high microsatellite instability and/or abnormal IHC for one or more of the mismatch repair genes are more likely to carry germline mutations. Germline testing can also be considered for patients with microsatellite stable tumors but high clinical suspicion for Lynch syndrome. For patients less likely to have Lynch syndrome, but have tumors deficient in MLH1 and PMS2 on IHC, somatic BRAF and MLH1 promoter hypermethylation testing may be considered prior to germline testing for *MLH1* and *PMS2*. Pre- and post-test genetic counseling is recommended for germline testing.



Methodology

***MLH1, MSH2, MSH6,* and *PMS2* 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 for the MLH1, MSH2, MSH6 genes. For PMS2, long range amplification is performed to avoid pseudogene homology regions, followed by Sanger sequencing. Targeted testing for known familial mutation is performed by Sanger sequencing.

***MLH1, MSH2, MSH6, PMS2, and EPCAM* MLPA analysis for large deletions or duplications:** SALSA Multiplex Ligation-Dependent Probe Amplification (MLPA) assays are used for exon-level detection of large deletions and duplications of the *MLH1*, *MSH2*, *MSH6*, *PMS2, and EPCAM* genes. Collectively, these probemixes cover all 19 exons of the *MLH1* gene, all 16 exons of the *MSH2* gene, all 10 exons of *MSH6*, exons 8, 9, and 3' UTR of *EPCAM*, and exons 1, 2, 5-12 of the *PMS2* gene.

Limitations

Sequence analysis does not detect mutations located in regions of the genes that are not analyzed (untranslated regions, intron regions other than the splice junctions). The method also will not detect gross genetic alterations including most duplications, inversions, or deletions. Some sequence alterations that may be detected will be of unknown clinical significance. Additionally, there are a number of established *PMS2* pseudogenes. The *PMS2* assay was designed 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.

MLPA assays designed to detect deletions/duplications of one or more exons. 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. Due to pseudogene interference, the deletion and duplication analysis of exons 3, 4, 13-15 of the *PMS2* gene

is excluded. Additionally, *PMS2* gene conversion has been reported to occur, which could interfere with the interpretation of certain deletions and duplications.
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) Providing relevant clinical and family history information/pedigree is recommended.

Order Codes: Germline Tests	***CPT Codes***	***TAT***
HNPCC-COMP (MLH1, MSH2, MSH6, PMS2: Seq+MLPA, EPCAM: MLPA)	81292, 81294, 81295, 81297, 81298, 81300, 81317, 81319, 81403, G0452(x2)	4 wks
MLH1-SEQ (MLH1 gene, full gene sequencing by NGS)	81292, G0452	3 wks
MSH2-SEQ (MSH2 gene, full gene sequencing by NGS)	81295, G0452	3 wks
MSH6-SEQ (MSH6 gene, full gene sequencing by NGS)	81298, G0452	3 wks
PMS2-SEQ (PMS2 gene, full gene sequencing)	81317, G0452	3 wks
MLH1-CAS (MLH1 gene, targeted mutation analysis)	81293, G0452	2 wks
MSH2-CAS (MSH2 gene, targeted mutation analysis)	81296, G0452	2 wks
MSH6-CAS (MSH6 gene, targeted mutation analysis)	81299, G0452	2 wks
PMS2-CAS (PMS2 gene, targeted mutation analysis)	81318, G0452	2 wks
MLH1-DEL (MLH1 gene, MLPA analysis)	81294, G0452	3 wks
MLH1-DEL-CAS (MLH1 gene, MLPA analysis, known deletions/duplications)	81294, G0452	3 wks
MSH2-DEL (MSH2 gene, MLPA analysis)	81297, G0452	3 wks
MSH2-DEL-CAS (MSH2 gene, MLPA analysis, known deletions/duplications)	81297, G0452	3 wks
MSH6-DEL (MSH6 gene, MLPA analysis)	81300, G0452	3 wks
MSH6-DEL-CAS (MSH6 gene, MLPA analysis, known deletions/duplications)	81300, G0452	3 wks
PMS2-DEL (PMS2 gene, MLPA analysis)	81319, G0452	3 wks
PMS2-DEL-CAS (PMS2 gene, MLPA analysis, known deletions/duplications)	81319, G0452	3 wks

Order Codes: Complementary tests in tumor	***CPT Codes***	***TAT***
IHC-HNPCC (HNPCC Immunohistochemistry- MLH1, MSH2, MSH6, PMS2)	88342(x4)	2 wks
MSI-P (Microsatellite Instability of tumor)	81301, 88381, G0452	1 wk
BRAF-NGS (Targeted analysis for 77 cancer hotspot mutations (including V600E) in the BRAF gene by next generation sequencing)	81210, 88381, G0452	2 wks
MLH1-METH (MLH1 gene, Somatic Promoter Methylation analysis)	81479, 88381, G0452	3 wks

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References

1. Kohlmann W, Gruber SB. Lynch Syndrome. In: Pagon RA, Adam MP, Ardinger HH, et al., eds. *GeneReviews(R)*. Seattle (WA)1993.
2. Miyaki M, Konishi M, Tanaka K, et al. Germline mutation of MSH6 as the cause of hereditary nonpolyposis colorectal cancer. *Nat Genet.* Nov 1997;17(3):271-272.
3. Plaschke J, Engel C, Kruger S, et al. Lower incidence of colorectal cancer and later age of disease onset in 27 families with pathogenic MSH6 germline mutations compared with families with MLH1 or MSH2 mutations: the German Hereditary Nonpolyposis Colorectal Cancer Consortium. *J Clin Oncol.* Nov 15 2004;22(22):4486-4494.
4. Berends MJ, Wu Y, Sijmons RH, et al. Molecular and clinical characteristics of MSH6 variants: an analysis of 25 index carriers of a germline variant. *Am J Hum Genet.* Jan 2002;70(1):26-37.
5. Wu Y, Berends MJ, Mensink RG, et al. Association of hereditary nonpolyposis colorectal cancer-related tumors displaying low microsatellite instability with MSH6 germline mutations. *Am J Hum Genet.* Nov 1999;65(5):1291-1298.
6. Niessen RC, Kleibeuker JH, Westers H, et al. PMS2 involvement in patients suspected of Lynch syndrome. *Genes Chromosomes Cancer.* Apr 2009;48(4):322-329.
7. Senter L, Clendenning M, Sotamaa K, et al. The clinical phenotype of Lynch syndrome due to germ-line PMS2 mutations. *Gastroenterology.* Aug 2008;135(2):419-428.
8. Bakry D, Aronson M, Durno C, et al. Genetic and clinical determinants of constitutional mismatch repair deficiency syndrome: report from the constitutional mismatch repair deficiency consortium. *European journal of cancer.* Mar 2014;50(5):987-996.
9. Jackson CC, Holter S, Pollett A, et al. Cafe-au-lait macules and pediatric malignancy caused by biallelic mutations in the DNA mismatch repair (MMR) gene PMS2. *Pediatric blood & cancer.* Jun 2008;50(6):1268-1270.
10. Agostini M, Tibiletti MG, Lucci-Cordisco E, et al. Two PMS2 mutations in a Turcot syndrome family with small bowel cancers. *The American journal of gastroenterology.* Aug 2005;100(8):1886-1891.
11. van der Klift H, Wijnen J, Wagner A, et al. Molecular characterization of the spectrum of genomic deletions in the mismatch repair genes MSH2, MLH1, MSH6, and PMS2 responsible for hereditary nonpolyposis colorectal cancer (HNPCC). *Genes Chromosomes Cancer.* Oct 2005;44(2):123-138.
12. Vaughn CP, Robles J, Swensen JJ, et al. Clinical analysis of PMS2: mutation detection and avoidance of pseudogenes. *Hum Mutat.* May 2010;31(5):588-593.
13. Taylor CF, Charlton RS, Burn J, Sheridan E, Taylor GR. Genomic deletions in MSH2 or MLH1 are a frequent cause of hereditary non-polyposis colorectal cancer: identification of novel and recurrent deletions by MLPA. *Hum Mutat.* Dec 2003;22(6):428-433.
14. Kovacs ME, Papp J, Szentirmay Z, Otto S, Olah E. Deletions removing the last exon of TACSTD1 constitute a distinct class of mutations predisposing to Lynch syndrome. *Hum Mutat.* Feb 2009;30(2):197-203.
15. Ligtenberg MJ, Kuiper RP, Chan TL, et al. Heritable somatic methylation and inactivation of MSH2 in families with Lynch syndrome due to deletion of the 3' exons of TACSTD1. *Nat Genet.* Jan 2009;41(1):112-117.