

Lynch Syndrome/Hereditary Nonpolyposis Colorectal Cancer (*MLH1*, *MSH2*, *MSH6*, and *PMS2*) Sequencing and Deletion/Duplication

FOR DIAGNOSTIC OR PRESYMPTOMATIC TESTING FOR LYNCH SYNDROME

Disease Overview

- In the United States, colorectal cancer is the third most common form of cancer; individuals have a 6% lifetime risk of developing this disease.
- Most colorectal cancer is caused by somatic mutations and is not hereditary.
- Hereditary nonpolyposis colorectal cancer (HNPCC) was originally diagnosed in individuals meeting Amsterdam Clinical Criteria, or by identification of a pathogenic germline mutation in a mismatch repair gene.
- Although HNPCC and Lynch syndrome were formerly synonymous, Lynch syndrome is currently defined as the subclass of individuals with germline mismatch repair gene mutations.
- Lynch syndrome characteristics include increased risk of colorectal cancer and extra-colonic cancers, including cancer of the endometrium, renal pelvis, ureter, ovary, stomach, small intestine, and hepatobiliary tract.
- Lynch syndrome-associated tumors commonly exhibit microsatellite instability (MSI), a contraction or expansion of short nucleotide repeats due to defective DNA mismatch repair.
- Fifteen percent of sporadic colon cancers are also MSI-high (MSI-H), mostly due to acquired methylation of the *MLH1* gene. Sporadic MSI-H cancers are often associated with a *BRAF* V600E gene mutation. With rare exceptions, MSI-high tumors associated with *BRAF* V600E gene mutations are not associated with germline mismatch repair gene mutations.
- Individuals with MSI-stable colon cancers have a very low risk for germline mismatch repair gene mutations.
- Immunohistochemistry (IHC) testing of MSI-H tumors detects the presence or the absence of proteins produced by the different mismatch repair genes; thus, it may be useful as a surrogate test for MSI and also in determining the specific mismatch repair gene to be evaluated. Occasionally, a pathogenic mismatch repair gene mutation does not lead to lack of protein production, leading to a false-negative result.
- Two additional syndromes with Lynch syndrome-like findings may also have germline mismatch repair gene mutations.
 - Muir-Torre syndrome: sebaceous neoplasms of the skin and internal malignancies typical of Lynch syndrome
 - Turcot syndrome: central nervous system tumors and colorectal adenomas or cancer

Epidemiology

HNPCC accounts for about 2% of colorectal and endometrial cancers.

Genetics

- Lynch syndrome occurs due to a pathogenic germline mutation in one of the following mismatch repair genes: *MLH1*, *MSH2*, *MSH6*, or *PMS2*.
- Inheritance is autosomal dominant.
- Penetrance is 80% for colorectal cancer.
- Germline mutations in the *MLH1* and the *MSH2* genes account for 90% of Lynch syndrome, while mutations in the *MSH6* and the *PMS2* genes are responsible for approximately 10%.
- Germline deletions of the 3' region of the *EPCAM* gene cause transcriptional readthrough, resulting in *MSH2* silencing by hypermethylation. Among individuals suspected of having Lynch syndrome, the frequency of somatic *MSH2* promoter hypermethylation due to large 3' *EPCAM* deletions is 0.9%.

Indications for Ordering

- This test is recommended for individuals who meet the following, updated Bethesda guidelines, especially those with MSI-H, *BRAF* V600E-negative tumors:
 - Colorectal cancer diagnosed in an individual younger than 50 years old
 - Presence of synchronous or metachronous colorectal tumors, or other HNPCC-associated tumors, regardless of age
 - Colorectal cancer with MSI-H histology (presence of tumor-infiltrating lymphocytes, Crohn-like lymphocytic reaction, mucinous/signet-ring differentiation, or medullary growth pattern), diagnosed in an individual younger than 60 years old
 - Colorectal cancer diagnosed in one or more first-degree relatives with an HNPCC-related tumor, with one cancer diagnosed before age 50
 - Colorectal cancer diagnosed in two or more first- or second-degree relatives of any age
 - Individuals with Muir-Torre or Turcot syndrome (especially when the brain tumor is a glioblastoma)

Additional Ordering Notes

- IHC on tumor tissue is highly recommended prior to ordering mismatch repair gene sequencing.
- To evaluate family members of individuals with a known Lynch syndrome mutation, order targeted testing for the familial mutation HNPCC/Lynch Syndrome, Family Specific Mutation (ARUP test# 2001961), as this is more cost-effective than full gene sequencing.
- For optimal test interpretation, please complete the “Lynch Syndrome Patient Information Form,” providing tumor microsatellite instability results (preferably including immunohistochemistry), patient age, tumor type, and family history of Lynch syndrome-associated tumors.

Contraindications

- Testing asymptomatic individuals under the age of 18 (unless a relative has been diagnosed with a Lynch syndrome-associated cancer before age 28)
- Prenatal testing

Interpretation

- A positive result means a pathogenic mutation in the *MLH1*, *MSH2*, *MSH6*, or *PMS2* gene was detected that is predictive of Lynch syndrome.
- A negative result does not rule out Lynch syndrome, due to the possibility of an undetectable mutation in the specific gene analyzed or a mutation in another mismatch repair gene.
- An uncertain result means that although a gene mutation was detected, it is not certain whether it is pathogenic or benign. Medical management should rely on clinical findings, family history, and/or testing of other mismatch repair genes.

Methodology

- Analysis of each specific mismatch gene requested involves:
- Bidirectional sequencing of the entire coding region and intron-exon borders and
- multiplex ligation-dependent probe amplification (MLPA) to detect large deletions or duplications.
- Large 3' *EPCAM* gene deletions are simultaneously detected by MLPA analysis of *MSH2*.
- PMS2* testing requires using a newly designed MLPA kit to detect deletions in both *PMS2* and *PMS2CL* (the *PMS2* pseudogene).

Large *PMS2* deletions can be distinguished from *PMS2CL* by using reference samples with known copy numbers of variants and pairing the MLPA and sequencing results for *PMS2* and *PMS2CL*. This recent, innovative methodology now allows for the detection of large *PMS2* deletions in exons where deletion detection was previously not possible (exons 11, 12, 13, 14, and 15).

- Analytical sensitivity and specificity are 99% for the *MLH1*, *MSH2*, *MSH6*, and *PMS2* genes.
- The clinical sensitivity for Lynch syndrome is approximately 45% by testing either the *MLH1* or the *MSH2* genes, and 5% by testing either the *MSH6* or the *PMS2* gene.

Limitations

- Mutations in intronic or regulatory regions will not be identified.
- Gene variants of uncertain significance may be detected.
- Breakpoints of large deletions/duplications will not be determined.
- Deep intronic or promoter mutations of the *MLH1*, *MSH2*, *MSH6*, and *PMS2* genes will not be detected.
- Rare diagnostic errors may occur due to primer or probe site mutations.

Related Tests

- HNPCC/Lynch Syndrome, Microsatellite Instability by PCR (0051740)
- Mismatch Repair by IHC (0049302)
- BRAF V600E Mutation with Reflex to MLH1 Promoter Methylation, Paraffin (0051750)

References

- Aaltonen LA, et al. Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. *N Engl J Med.* 1998;338:1481–7.
- Aarnio M, et al. Life-time risk of different cancers in hereditary nonpolyposis colorectal cancer (HNPCC) syndrome. *Int J Cancer.* 1995;64:430–3.
- Samowitz WS, et al. The colon cancer burden of genetically defined hereditary nonpolyposis colon cancer. *Gastroenterology.* 2001;121:830–8.
- Umar A, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J Natl Cancer Inst.* 2004;96:261–8.

Test Information

0051650	HNPCC/Lynch Syndrome (<i>MLH1</i>) Sequencing and Deletion/Duplication
0051654	HNPCC/Lynch Syndrome (<i>MSH2</i>) Sequencing and Deletion/Duplication
0051656	HNPCC/Lynch Syndrome (<i>MSH6</i>) Sequencing and Deletion/Duplication
0051737	HNPCC/Lynch Syndrome (<i>PMS2</i>) Sequencing and Deletion/Duplication
2001728	HNPCC/Lynch Syndrome Deletion/Duplication
2001961	Familial Mutation, Targeted Sequencing

For specific collection, transport, and testing information, refer to the ARUP website at www.aruplab.com.

For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

AUTHORS

Chris Miller, MS, LCGC
Wade Samowitz, MD
Jeffrey J. Swensen, PhD
Cecily Vaughn, PhD