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Lynch Syndrome/Hereditary Nonpolyposis Colorectal Cancer

Colorectal cancer (CRC) exhibits the characteristics of familial clustering in ~10-15% of cases. The most common cause of hereditary CRC is Lynch syndrome (LS), also known as hereditary nonpolyposis colorectal cancer (HNPCC). LS is caused by a germline mutation in one of the genes within the DNA mismatch repair (MMR) system.

INDICATIONS FOR ORDERING

Microsatellite instability (MSI) and/or immunohistochemistry (IHC) tumor analyses

- Universal screening for Lynch syndrome (LS) in all individuals with newly diagnosed colorectal cancer (CRC) (NCCN, 2018)
- Screening for LS in individuals with endometrial cancer (ASCO, 2015)

Germline mismatch repair (MMR) gene testing

- Order following abnormal screening test result
- Confirms diagnosis of LS

DISEASE OVERVIEW

Incidence

- Accounts for 2-4% of CRC and ~1-2% of endometrial cancers (NCCN, 2018; Chadwick, 2001)
- Most common hereditary CRC syndrome
- Most common cause of hereditary endometrial cancer

Risk Estimates

The following lifetime risk estimates apply to individuals with *MLH1* and *MSH2* pathogenic variants; risks for variants in *MSH6* or *PMS2* may be lower (NCCN, 2018)

- CRC – 52-82%
- Endometrial – 25-60%
- Prostate – ~30%
- Ovarian – 11-24%
- Gastric – 6-13%
- Hepatobiliary tract – 1-4%
- Urinary tract – 1-7%
- Small bowel – 3-6%
- Brain/central nervous system – 1-3%
- Sebaceous neoplasms – 1-9%
- Pancreatic – 1-6% (*MLH1* and *MSH2* only)

GENETICS

Genes Tested

MLH1, *MSH2*, *MSH6*, *PMS2,* and *EPCAM*

Inheritance – autosomal dominant

TEST INTERPRETATION

TESTS TO CONSIDER

Screening Studies (Requires Pathological Tissue)

Mismatch Repair by Immunohistochemistry 0049302
Method: Qualitative Immunohistochemistry

- First-line screening test for LS
- Highly recommended prior to ordering germline MMR gene testing
  - Directs subsequent genetic diagnostic testing

Microsatellite Instability (MSI), HNPCC/Lynch Syndrome, by PCR 0051740
Method: Polymerase Chain Reaction/Fragment Analysis

- First-line screening test for LS
- Directs subsequent genetic diagnostic testing for LS

Mismatch Repair by Immunohistochemistry with Reflex to BRAF Codon 600 Mutation and MLH1 Promoter Methylation 2002327
Method: Qualitative Immunohistochemistry/Qualitative Real-time Polymerase Chain Reaction

- Preferred screening test for LS in individuals with CRC
- If MLH1 IHC is abnormal, evaluations of *BRAF* codon 600 and, possibly, *MLH1* methylation are performed
- Definitive diagnosis of LS requires additional targeted MMR germline molecular studies
- Do not use in endometrial cancer

BRAF Codon 600 Mutation Detection with Reflex to MLH1 Promoter Methylation 0051750
Method: Polymerase Chain Reaction/Pyrosequencing

- Recommended reflex test to differentiate between LS and sporadic CRC in tumors showing loss of MLH1

Microsatellite Instability (MSI), HNPCC/Lynch Syndrome, by PCR

- Sensitivity/specificity
  - Clinical sensitivity – 90% (NCCN, 2018)
  - Analytical sensitivity/specificity – >99%
- Results
  - High MSI (MSI-H)
    - MMR deficiency, either sporadic or LS
  - Indeterminate MSI (MSI-I)
    - Instability in even 1 mononucleotide repeat can be associated with LS
    - Follow-up IHC studies are recommended
  - Microsatellite stable (MSS)
    - LS unlikely
- Limitations
  - 10-15% of sporadic CRCs are also MSI-H (NCCN, 2018)
  - Preoperative chemoradiation of rectal cancer
    - May complicate IHC interpretation and/or decrease tumor mass
    - May make MSI testing difficult
    - Evaluation of pretreatment biopsies will avoid this limitation
  - Screens for LS only and does not evaluate other hereditary causes of CRC or endometrial cancer

Mismatch Repair by IHC

- Clinical sensitivity
  - 90% (NCCN, 2018)
- Results
  - Normal - MMR proteins are normally expressed
    - MMR deficiency is unlikely
    - LS unlikely
  - Abnormal - MMR protein expression is abnormal
    - Loss of expression of 1 or more proteins is highly predictive of MMR deficiency
    - Absence of both MLH1 and PMS2
      - *MLH1* germline pathogenic variant is possible
      - Consider *MLH1* methylation ± *BRAF* V600E studies
      - If methylation and *BRAF* studies are negative, follow with *MLH1* germline genetic testing
    - Absence of PMS2 only
      - *PMS2* germline pathogenic variant likely
      - Consider *PMS2* germline testing
      - If *PMS2* testing does not identify a germline pathogenic variant, consider *MLH1* germline testing
    - Absence of both MSH2 and MSH6
      - *MSH2* germline pathogenic variant likely
      - Consider *MSH2* germline testing
    - Absence of MSH6 only
      - *MSH6* germline pathogenic variant likely
      - Consider *MSH6* germline testing
      - If *MSH6* testing does not identify a germline pathogenic variant, consider *MSH2* germline testing
- Limitations
  - ~10% of individuals with LS will have IHC tests that show normal staining of the MMR proteins
  - Because the correlation of MSI with IHC is not 100%, direct testing of MSI by PCR may be helpful
  - Screens for LS only and does not evaluate other hereditary causes of CRC or endometrial cancer

- If no *BRAF* variant is detected, *MLH1* promoter methylation is evaluated

Mismatch Repair by Immunohistochemistry with Reflex to MLH1 Promoter Methylation 2005270
Method: Qualitative Immunohistochemistry/Qualitative Real-time Polymerase Chain Reaction

- Preferred reflex screening test for LS in non-CRC tumors (eg, endometrial carcinoma)
- If *MLH1* expression is lost, *MLH1* methylation is performed

MLH1 Promoter Methylation, Paraffin 2002499
Method: Real-Time Polymerase Chain Reaction/Fluorescence Resonance Energy Transfer

Recommended test to distinguish between LS and sporadic non-CRC tumors with loss of MLH1

Diagnostic Germline Genetic Studies

- Specimen – peripheral blood
- Germline genetic testing is available for all 4 MMR genes known to cause LS, either separately or as part of the hereditary gastrointestinal (GI) cancer panel (see Related Tests)

HNPCC/Lynch Syndrome (MLH1) Sequencing and Deletion/Duplication 0051650
Method: Polymerase Chain Reaction/Sequencing/Multiplex Ligation-dependent Probe Amplification

- Detect germline *MLH1* variants
- Use in MMR-deficient carcinoma with suggestive IHC results (loss of MLH1 and PMS2 proteins), negative for the *BRAF* codon 600 pathogenic variant, and with normal *MLH1* promoter methylation studies

HNPCC/Lynch Syndrome (MSH2) Sequencing and Deletion/Duplication 0051654
Method: Polymerase Chain Reaction/Sequencing/Multiplex Ligation-dependent Probe Amplification

- Detect germline *MSH2* variants
- Use in MMR-deficient carcinoma with suggestive IHC results (loss of MSH2 and MSH6 proteins)
- Includes evaluation of *EPCAM* exon 9 deletions and 10 Mb inversion of *MSH2* exons 1-7

BRAF Codon 600 Mutation Detection with Reflex to _MLH1_ Promoter Methylation

- Analytical sensitivity
  - Methylation levels >10% are reported as positive
- Results
  - _BRAF_ V600E detected
    - Presence in MMR-deficient CRC indicates the tumor is probably sporadic and not associated with LS
    - Further germline testing not typically indicated
  - _MLH1_ promoter methylation detected
    - Presence in an MSI CRC indicates the tumor is probably sporadic and not associated with LS
    - Further germline testing not typically indicated
  - No variants detected
    - In MSI-H tumors with loss of MLH1 protein by IHC, _MLH1_ germline testing indicated

Germline Genetic Studies (_MLH1, MSH2, MSH6_, _PMS2,_ or _EPCAM_)

- Sensitivity/specificity
  - Proportion of LS attributed to pathogenic variants in specific MMR gene
    - _MLH1_ – 50% (Smith 2016)
    - _MSH2_ – 40% (Smith 2016)
    - _MSH6_ – 7-10% (Miyaki 1997; Berends 2002; Petomaki 2003)
    - _PMS2_ – <5% (Senter 2008)
    - _EPCAM_ – ~1-3% (Kuiper 2011)
  - Analytical sensitivity/specificity – 99%
- Results
  - Positive – 1 pathogenic variant detected
    - Predicted to be causative for LS
  - Negative – no pathogenic variants detected
    - Diagnosis of LS unlikely, but not excluded
  - Inconclusive – variant detected, but whether it is benign or pathogenic is unknown
- Limitations
  - Not evaluated
    - Regulatory region and deep intronic variants
    - Sequence variants and large deletion/duplications in genes other than _MLH1, MSH2, MSH6,_ and _PMS2_
    - Sequence variants in _EPCAM_
    - Large deletions/duplications in _EPCAM_, other than exon 9
    - Large gene inversions, other than the _MSH2_ 10 Mb exons 1-7 inversion
    - Causes of hereditary CRC or endometrial cancer other than LS
  - Diagnostic errors can occur due to rare sequence variations

HNPCC/Lynch Syndrome (MSH6) Sequencing and Deletion/Duplication 0051656
Method: Polymerase Chain Reaction/Sequencing/Multiplex Ligation-dependent Probe Amplification

- Detect germline _MSH6_ variants
- Use in MMR-deficient carcinoma with suggestive IHC results (isolated loss of MSH6 protein)

HNPCC/Lynch Syndrome (PMS2) Sequencing and Deletion/Duplication 0051737
Method: Polymerase Chain Reaction/Sequencing/Multiplex Ligation-dependent Probe Amplification

- Detect germline _PMS2_ variants
- Use in MMR-deficient carcinoma with suggestive IHC results (isolated loss of PMS2 protein)

HNPCC/Lynch Syndrome Deletion/Duplication 2001728
Method: Polymerase Chain Reaction/Multiplex Ligation-dependent Probe Amplification

- Order if sequencing studies have been performed previously at another laboratory
- Order if there is a known familial deletion or duplication
- Both sequencing and deletion/duplication testing are necessary to detect all pathogenic variants in MMR genes

Familial Mutation, Targeted Sequencing 2001961
Method: Polymerase Chain Reaction/Sequencing

Useful when a pathogenic familial variant identifiable by sequencing is known

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RELATED TESTS

Hereditary Gastrointestinal Cancer Panel, Sequencing and Deletion/Duplication 2013449
Method: Massively Parallel Sequencing/Exonic Oligonucleotide-based CGH Microarray/Sequencing/Multiplex Ligation-dependent Probe Amplification

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