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 LS in all individuals with newly diagnosed CRC[1]
- Universal screening for LS in individuals with endometrial cancer[2]

Germline 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[2,3]
- 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[1,2]

- 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)

Tests to Consider

Screening Studies (Requires Pathological Tissue)

Mismatch Repair by Immunohistochemistry 0049302

Method: Qualitative Immunohistochemistry

- First-line screening test for newly diagnosed CRC, endometrial carcinoma, and LS
- Highly recommended prior to ordering germline MMR gene testing
  - Directs subsequent genetic diagnostic testing
- Testing for CRC and other solid tumors to qualify patients for certain immune checkpoint inhibitor treatment

Microsatellite Instability (MSI), HNPCC/Lynch Syndrome, by PCR 0051740

Method: Polymerase Chain Reaction/Fragment Analysis

- First-line screening test for newly diagnosed CRC, endometrial carcinoma, and LS
- Directs subsequent genetic diagnostic testing for LS
- Testing for CRC and other solid tumors to qualify patients for certain immune checkpoint inhibitor treatment

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

- Screening for HNPCC/LS (NCCN, Colorectal, 2018)

Genetics

Genes Tested

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

Inheritance – autosomal dominant

Test Interpretation

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

- Sensitivity/specificity
  - Clinical sensitivity – 90%[1,2]
  - 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[1,2]
  - 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%[1,2]
- 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
- 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
- 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

- 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

        - 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

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%[4]
        - *MSH2* – 40%[4]

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

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

        - *MSH6* – 7-10%[5,6,7]
        - *PMS2* – <5%[8]
        - *EPCAM* – ~1-3%[9]
    - 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

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

See Related Tests

References

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Related Information

Colorectal Cancer
Lynch Syndrome - Hereditary Nonpolyposis Colorectal Cancer (HNPCC)
Lynch Syndrome (HNPCC) Testing Algorithm

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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