

Protocol

Genetic Testing for Lynch Syndrome and Other Inherited Colon Cancer Syndromes

(20408)

(Formerly Genetic Testing for FAP and Lynch Syndrome)

Medical Benefit		Effective Date: 04/01/19	Next Review Date: 11/19
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Preauthorization is required.

The following protocol contains medical necessity criteria that apply for this service. The criteria are also applicable to services provided in the local Medicare Advantage operating area for those members, unless separate Medicare Advantage criteria are indicated. If the criteria are not met, reimbursement will be denied and the patient cannot be billed. Please note that payment for covered services is subject to eligibility and the limitations noted in the patient's contract at the time the services are rendered.

Populations	Interventions	Comparators	Outcomes
Individuals: • Who are suspected of attenuated FAP, MAP and Lynch Syndrome, or are at-risk relatives of patients with FAP	Interventions of interest are: • Genetic testing for APC	Comparators of interest are: • No genetic testing	Relevant outcomes include: • Overall survival • Disease-specific survival • Test accuracy • Test validity
Individuals: • Who are suspected of attenuated FAP, MAP and Lynch Syndrome	Interventions of interest are: • Genetic testing for MUTYH after a negative APC test result	Comparators of interest are: • No genetic testing	Relevant outcomes include: • Overall survival • Disease-specific survival • Test accuracy • Test validity
Individuals: • Who are suspected attenuated of FAP, MAP, and Lynch Syndrome; CRC; or endometrial cancer and first-degree relative with Lynch	Interventions of interest are: • Genetic testing for MMR genes	Comparators of interest are: • No genetic testing	Relevant outcomes include: • Overall survival • Disease-specific survival • Test accuracy • Test validity
Individuals: • Who are at-risk relatives of patients with Lynch or family history meeting appropriate criteria, but do not have CRC	Interventions of interest are: • Genetic testing for MMR genes	Comparators of interest are: • No genetic testing	Relevant outcomes include: • Overall survival • Disease-specific survival • Test accuracy • Test validity
Individuals: • Who warrant Lynch testing, screen negative on MMR testing, but positive for MSI and lack MSH2 protein expression	Interventions of interest are: • Genetic testing for EPCAM variants	Comparators of interest are: • No genetic testing	Relevant outcomes include: • Overall survival • Disease-specific survival • Test accuracy • Test validity

Populations	Interventions	Comparators	Outcomes
Individuals: <ul style="list-style-type: none"> • With CRC in whom MLH1 protein is not expressed on immunohistochemical analysis 	Interventions of interest are: <ul style="list-style-type: none"> • Genetic testing for BRAF V600E or MLH1 promoter methylation 	Comparators of interest are: <ul style="list-style-type: none"> • No genetic testing 	Relevant outcomes include: <ul style="list-style-type: none"> • Overall survival • Disease-specific survival • Test accuracy • Test validity
Individuals: <ul style="list-style-type: none"> • Who are suspected of juvenile polyposis syndrome or are at-risk relatives of patients suspected of or diagnosed with JPS 	Interventions of interest are: <ul style="list-style-type: none"> • Genetic testing for SMAD4 and BMPR1A genes 	Comparators of interest are: <ul style="list-style-type: none"> • No genetic testing 	Relevant outcomes include: <ul style="list-style-type: none"> • Overall survival • Disease-specific survival • Test accuracy • Test validity
Individuals: <ul style="list-style-type: none"> • Who are suspected of Peutz-Jeghers syndrome or are at-risk relatives of patients suspected of or diagnosed with PJS 	Interventions of interest are: <ul style="list-style-type: none"> • Genetic testing for STK11 gene 	Comparators of interest are: <ul style="list-style-type: none"> • No genetic testing 	Relevant outcomes include: <ul style="list-style-type: none"> • Overall survival • Disease-specific survival • Test accuracy • Test validity

CRC: colorectal cancer; FAP: familial adenomatous polyposis; MAP: MUTYH-associated polyposis; MMR: mismatch repair; MSI: microsatellite instability.

DESCRIPTION

Genetic testing is available for both those with and those at risk for various types of hereditary cancer. This protocol evaluates genetic testing for hereditary colorectal cancer and polyposis syndromes, including familial adenomatous polyposis (FAP), Lynch syndrome (formerly known as hereditary nonpolyposis colorectal cancer), MUTYH-associated polyposis (MAP), Lynch syndrome–related endometrial cancer, juvenile polyposis syndrome, and Peutz-Jeghers syndrome.

SUMMARY OF EVIDENCE

For individuals who are suspected of attenuated FAP, MAP, and Lynch syndrome who receive genetic testing for APC, or are at-risk relatives of patients with FAP who receive genetic testing for MUTYH after a negative APC test result, the evidence includes a TEC Assessment. Relevant outcomes are overall survival, disease-specific survival, and test accuracy and validity. For patients with an APC variant, enhanced surveillance and/or prophylactic treatment will reduce the future incidence of colon cancer and improve health outcomes. A related familial polyposis syndrome, MAP syndrome, is associated with variants in the MUTYH gene. Testing for this genetic variant is necessary when the differential diagnosis includes both FAP and MAP because distinguishing between the two leads to different management strategies. Depending on presentation, Lynch syndrome may be part of the same differential diagnosis. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals who (1) are suspected of attenuated FAP, MAP, and Lynch syndrome, or (2) have colon cancer, or (3) have endometrial cancer and a first-degree relative diagnosed with a Lynch-associated cancer, or (4) are at-risk relatives of patients with Lynch syndrome, or (5) are without colon cancer but with a family history meeting the Amsterdam or Revised Bethesda criteria who receive genetic testing for mismatch repair (MMR) genes, the evidence includes an Agency for Healthcare Research and Quality report, a supplemental assessment to that report by the Evaluation of Genomic Applications in Practice and Prevention Working Group, and an Evaluation

of Genomic Applications in Practice and Prevention recommendation for genetic testing in colorectal cancer (CRC). Relevant outcomes are overall survival, disease-specific survival, and test accuracy and validity. A chain of evidence from well-designed experimental nonrandomized studies is adequate to demonstrate the clinical utility of testing unaffected (without cancer) first- and second-degree relatives of patients with Lynch syndrome who have a known variant in an MMR gene, in that counseling has been shown to influence testing and surveillance choices among unaffected family members of Lynch syndrome patients. One long-term, nonrandomized controlled study and a cohort study of Lynch syndrome family members found significant reductions in CRC among those who followed recommended colonic surveillance. A positive genetic test for an MMR variant can also lead to changes in the management of other Lynch syndrome malignancies. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals who warrant Lynch testing, screen negative on MMR testing, but positive for microsatellite instability and lack MSH2 protein expression who receive genetic testing for EPCAM variants, the evidence includes variant prevalence studies and case series. Relevant outcomes are overall survival, disease-specific survival, and test accuracy and validity. Studies have shown an association between EPCAM variants and Lynch-like disease in families, and the cumulative risk for CRC is similar to carriers of an MSH2 variant. Identification of an EPCAM variant could lead to changes in management that improve health outcomes. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals who have CRC in whom MLH1 protein is not expressed on immunohistochemical analysis who receive genetic testing for BRAF V600E or MLH1 promoter methylation, the evidence includes case series. Relevant outcomes are overall survival, disease-specific survival, and test accuracy and validity. Studies have shown, with high sensitivity and specificity, an association between BRAF V600E variant and MLH1 promoter methylation with sporadic CRC. Therefore, this type of testing could eliminate the need for further genetic testing or counseling for Lynch syndrome. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals who (1) are suspected of juvenile polyposis syndrome (JPS) or Peutz-Jeghers syndrome (PJS) or (2) are at-risk relatives of patients suspected of or diagnosed with JPS or PJS who receive genetic testing for SMAD4, BMPR1A, or STK11 genes, respectively, the evidence includes multiple observational studies. Relevant outcomes are overall survival, disease-specific survival, and test accuracy and validity. Studies have shown, with high sensitivity and specificity, an association between SMAD4 and BMPR1A and STK11 variants with JPS and PJS, respectively. Direct evidence of clinical utility for genetic testing of a JPS or PJS is not available. Genetic testing may have clinical utility by avoiding burdensome and invasive endoscopic examinations, release from intensified screening program resulting in psychological relief, and may improve health outcomes by identifying currently unaffected at-risk family members who require intense surveillance or prophylactic colectomy. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

POLICY

GENETIC TESTING FOR FAP

Genetic testing for Familial Adenomatous Polyposis (FAP) by testing for APC gene variants may be considered **medically necessary** in ANY of the following:

- Individuals with greater than 20 colonic polyps; OR
- First-degree relatives of individuals with FAP or attenuated familial adenomatous polyposis (AFAP) and/or a known APC variant. Exceptions may be necessary in the case of a small family pedigree.

Genetic testing for Familial Adenomatous Polyposis (FAP) by testing for MUTYH gene variants may be considered **medically necessary** in ANY of the following:

- Individuals with personal history of adenomatous polyposis who have negative APC variant testing and a negative family history for adenomatous polyposis; OR
- Individuals with personal history of adenomatous polyposis whose family history is positive only for sibling(s); OR
- Asymptomatic siblings if his/her sibling has a known MYH polyposis; OR
- History of Desmoid tumor.

GENETIC TESTING FOR LYNCH SYNDROME (SEE POLICY GUIDELINES FOR RECOMMENDED TESTING PROTOCOL*)

Genetic testing for MMR gene variants in MLH1 and MSH2 genes to determine the carrier status or diagnosis of Lynch syndrome is considered **medically necessary** in ANY of the following:

- Individuals with colorectal cancer; OR
- Individuals with endometrial cancer and one first-degree relative diagnosed with a Lynch-associated cancer; OR
- Individuals without colorectal cancer but who have a first- or second-degree relative with a known MMR variant; OR
- At-risk relatives of Individuals with Lynch syndrome with a known MMR variant; OR
- Individuals without colorectal cancer determined to be at high risk when no affected family members have been tested for MMR variants. High risk is defined as meeting either Amsterdam II or Revised Bethesda Guidelines or with a significant predicted risk of LS on MMRpro, PREMM, or MMRpredict, the accepted computer prediction models.

Genetic testing for MSH6 and/or PMS2 gene variants may be considered **medically necessary** in high risk Individuals who do not have variants in either the MLH1 or MSH2 genes with ANY of the following:

- Individuals with colorectal cancer whose screening for MSI or IHC is positive but MLH1 or MSH2 are normal; OR
- Targeted variant MSH6 and/or PMS2 testing is established for family members (up to third-degree) of individuals with Lynch syndrome with an identified MSH6 and/or PMS2 gene variant; OR
- Individuals with endometrial cancer and one first-degree relative diagnosed with a Lynch-associated cancer who do not have variants in either the MLH1 or MSH2 genes.

Genetic testing for EPCAM variants may be considered **medically necessary** in individuals with colorectal cancer whose:

- Tumor tissue shows a high level of microsatellite instability and patient is negative for a germline variant in MSH2, MLH1, PMS2, and MSH6; OR
- Tumor tissue shows lack of *MSH2* expression by immunohistochemistry AND
 - Individual is negative for a germline variant in MSH2, MLH1, PMS2, and MSH6; OR
 - At-risk relatives of individuals with Lynch syndrome with a known EPCAM variant.

Genetic testing for BRAF V600E variants or *MLH1* promoter methylation may be considered **medically necessary** to exclude a diagnosis of Lynch syndrome when *MLH1 protein* is not expressed in a colorectal cancer on immunohistochemical (IHC) analysis.

GENTIC TESTING FOR JUVENILE POLYPOSIS SYNDROME

Genetic testing for SMAD4 and BMPR1A gene variants may be considered **medically necessary** when either of the following major criteria (solid bullets) is met:

- Patients with a clinical diagnosis of juvenile polyposis syndrome based on the presence of any one of the following:
 - at least three to five juvenile polyps in the colon
 - multiple juvenile polyps in other parts of the gastrointestinal tract
 - any number of juvenile polyps in a person with a known family history of juvenile polyps.
- At-risk relative of a patient suspected of or diagnosed with juvenile polyposis syndrome.

GENTIC TESTING FOR PEUTZ-JEGHERS SYNDROME

Genetic testing for STK11 gene variants may be considered **medically necessary** when either of the following major criteria (solid bullets) is met:

- Patients with a clinical diagnosis of Peutz-Jeghers syndrome based on the presence of any two of the following:
 - presence of two or more histologically confirmed Peutz-Jeghers polyps of the small intestine
 - characteristic mucocutaneous pigmentation of the mouth, lips, nose, eyes, genitalia, or fingers
 - family history of Peutz-Jeghers syndrome
- At-risk relative of a patient suspected of or diagnosed with Peutz-Jeghers syndrome.

Genetic Testing for FAP by testing for the APC gene variant in those with FAP diagnosed by clinical criteria is considered **not medically necessary**.

Genetic Testing for Lynch Syndrome using panels, total genome or total exome sequencing using next generation sequencing is considered **investigational**.

Genetic testing for all other gene variants for Lynch syndrome or colorectal cancer is considered **investigational**.

POLICY GUIDELINES

TESTING AT-RISK RELATIVES

Due to the high lifetime risk of cancer of most genetic syndromes discussed in this protocol, “at-risk relatives” primarily refers to first-degree relatives. However, some judgment must be permitted, e.g., in the case of a small family pedigree, when extended family members may need to be included in the testing strategy.

TARGETED FAMILIAL VARIANT TESTING

It is recommended that, when possible, initial genetic testing for FAP or Lynch syndrome be performed in an affected family member so that testing in unaffected family members can focus on the variant found in the affected family member.

In many cases, genetic testing for MUTYH gene variants should first target the specific variants Y165C and G382D, which account for more than 80% of variants in white populations, and subsequently proceed to sequencing only as necessary. However, in other ethnic populations, proceeding directly to sequencing is appropriate.

EVALUATION FOR LYNCH SYNDROME

For patients with CRC being evaluated for Lynch syndrome, either the microsatellite instability (MSI) test or the immunohistochemical (IHC) test with or without BRAF gene variant testing, should be used as an initial evaluation of tumor tissue before mismatch repair (MMR) gene analysis. Both tests are not necessary. Proceeding to MMR gene sequencing would depend on results of MSI or IHC testing. In particular, IHC testing may help direct which MMR gene likely contains a variant, if any, and may also provide additional information if MMR genetic testing is inconclusive.

When indicated, genetic sequencing for MMR gene variants should begin with MLH1 and MSH2 genes unless otherwise directed by the results of IHC testing. Standard sequencing methods will not detect large deletions or duplications; when MMR gene variants are expected based on IHC or MSI studies but none are found by standard sequencing, additional testing for large deletions or duplications is appropriate.

Several Clinical Laboratory Improvement Amendments (CLIA)-licensed clinical laboratories offer MMR gene variant testing for Lynch syndrome. For example, the GeneTests website, available online at:

(<https://www.ncbi.nlm.nih.gov/gtr/all/?term=lynch+syndrome>) lists laboratories that offer this service. In at least one laboratory, Lynch syndrome variant testing is packaged under a copyrighted name. The COLARIS® test (Myriad Genetic Laboratories) includes sequence analysis of MLH1, MSH2, MSH6 and PMS2; large rearrangement analysis for MLH1, MSH2, PMS2 and MSH6 large deletions and duplications; and analysis for large deletions in the EPCAM gene near MSH2. Note that there are two versions of this test, the COLARIS (excludes *PMS2* testing) and COLARIS Update (includes *PMS2* testing). Individualized tested (e.g., targeted testing for a family variant) can also be requested. The COLARIS^{PLUS} test includes full sequence analysis of the MLH1, MSH2, MSH6, PMS2 and MYH genes and rearrangement analysis of MLH1, MSH2, MSH6, MYH, and EPCAM using microarray comparative genomic hybridization analysis, and of PMS2 using multiplex ligation-dependent probe amplification analysis.

Similarly, GeneTests lists U.S.-based CLIA-licensed clinical laboratories that provide APC variant testing and those that provide MUTYH variant testing. The COLARIS AP test (Myriad Genetic Laboratories) includes DNA sequencing analysis of the APC and MUTYH genes as well as analysis of large rearrangements in the APC gene not detected by DNA sequencing.

The Amsterdam II Clinical Criteria (all criteria must be fulfilled) are the most stringent for defining families at high risk for Lynch Syndrome (Vasen et al, 1999):

- Three or more relatives with an associated cancer (CRC, or cancer of the endometrium, small intestine, ureter or renal pelvis);
- One should be a first-degree relative of the other two;
- Two or more successive generations affected;
- One or more relatives diagnosed before the age of 50 years;
- Familial adenomatous polyposis should be excluded in cases of CRC;
- Tumors should be verified by pathologic examination;
- Modifications:

- EITHER: very small families, which cannot be further expanded, can be considered to have hereditary nonpolyposis colorectal cancer (HNPCC) with only two CRCs in first-degree relatives if at least two generations have the cancer and at least one case of CRC was diagnosed by the age of 55 years;
- OR: in families with two first-degree relatives affected by CRC, the presence of a third relative with an unusual early-onset neoplasm or endometrial cancer is sufficient.

The Revised Bethesda Guidelines (fulfillment of any criterion meets guidelines) are less stringent than the Amsterdam criteria and are intended to increase the sensitivity of identifying at-risk families (Umar et al, 2004). The Bethesda guidelines are also considered more useful in identifying which patients with CRC should have their tumors tested for microsatellite instability and/or immunohistochemistry:

- CRC diagnosed in a patient who is younger than 50 years old;
- Presence of synchronous or metachronous CRC or other HNPCC-associated tumors*, regardless of age;
- CRC with high microsatellite instability histology diagnosed in a patient younger than 60 years old;
- CRC diagnosed in one or more first-degree relatives with a Lynch syndrome-associated tumor, with one of the cancers being diagnosed before 50 years of age;
- CRC diagnosed in two or more first or second-degree relatives with HNPCC-related tumors,* regardless of age.

* HNPCC-related tumors include colorectal, endometrial, stomach, ovarian, pancreas, ureter and renal pelvis, biliary tract, brain [usually glioblastoma as seen in Turcot syndrome], sebaceous gland adenomas and keratoacanthomas in Muir-Torre syndrome, and carcinoma of the small bowel.

Multiple risk prediction models that provide quantitative estimates of the likelihood of an MMR variant are available such MMRpro, PREMM5 (Kastrinos et al, 2017), or MMRpredict. National Comprehensive Cancer Network guidelines recommend (category 2A) testing for Lynch syndrome in individuals with a significant predicted risk of Lynch syndrome on these risk prediction models. The PREMM5 model is available at [PREMM 5 Model | index](#).

GENETICS NOMENCLATURE UPDATE

The Human Genome Variation Society nomenclature is used to report information on variants found in DNA and serves as an international standard in DNA diagnostics. It is being implemented for genetic testing medical evidence review updates starting in 2017 (see Table PG1). The Society's nomenclature is recommended by the Human Variome Project, the HUMAN Genome Organization, and by the Human Genome Variation Society itself.

The American College of Medical Genetics and Genomics and the Association for Molecular Pathology standards and guidelines for interpretation of sequence variants represent expert opinion from both organizations, in addition to the College of American Pathologists. These recommendations primarily apply to genetic tests used in clinical laboratories, including genotyping, single genes, panels, exomes, and genomes. Table PG2 shows the recommended standard terminology—"pathogenic," "likely pathogenic," "uncertain significance," "likely benign," and "benign"—to describe variants identified that cause Mendelian disorders.

Table PG1. Nomenclature to Report on Variants Found in DNA

Previous	Updated	Definition
Mutation	Disease-associated variant	Disease-associated change in the DNA sequence
	Variant	Change in the DNA sequence
	Familial variant	Disease-associated variant identified in a proband for use in subsequent targeted genetic testing in first-degree relatives

Table PG2. ACMG-AMP Standards and Guidelines for Variant Classification

Variant Classification	Definition
Pathogenic	Disease-causing change in the DNA sequence
Likely pathogenic	Likely disease-causing change in the DNA sequence
Variant of uncertain significance	Change in DNA sequence with uncertain effects on disease
Likely benign	Likely benign change in the DNA sequence
Benign	Benign change in the DNA sequence

ACMG: American College of Medical Genetics and Genomics; AMP: Association for Molecular Pathology.

GENETIC COUNSELING

Experts recommend formal genetic counseling for patients who are at risk for inherited disorders and who wish to undergo genetic testing. Interpreting the results of genetic tests and understanding risk factors can be difficult for some patients; genetic counseling helps individuals understand the impact of genetic testing, including the possible effects the test results could have on the individual or their family members. It should be noted that genetic counseling may alter the utilization of genetic testing substantially and may reduce inappropriate testing; further, genetic counseling should be performed by an individual with experience and expertise in genetic medicine and genetic testing methods.

MEDICARE ADVANTAGE

For Medicare Advantage, the above criteria apply but only for the member if they are personally afflicted by a colorectal cancer. Also for the tests to be considered **medically necessary** the results must be intended to be used in the management of the member, such as to determine the extent of surgical treatment, a change in surveillance schedule or other therapeutic management.

Microsatellite instability analysis (e.g., hereditary non-polyposis colorectal cancer, LYNCH syndrome) of markers for mismatch repair deficiency (e.g., BAT25, BAT26), is considered **medically necessary** in individuals who have colorectal cancer (CRC) diagnosed at less than or equal to 70 years of age, and those greater than 70 years who meet the revised Bethesda Lynch Syndrome (LS) guidelines to guide therapeutic decision-making.

Testing for variants in the APC gene is unlikely to impact therapeutic decision-making in the clinical management of the patient and is considered **not medically necessary**.

Testing of unaffected family members or other individuals is screening and **not medically necessary**.

BACKGROUND

HEREDITARY COLORECTAL CANCERS

Currently, two types of hereditary CRC are well-defined: FAP and Lynch syndrome (formerly hereditary non-polyposis CRC). Lynch syndrome has been implicated in some endometrial cancers as well.

FAP and Associated Variants

FAP typically develops by age 16 years and can be identified by the appearance of hundreds to thousands of characteristic, precancerous colon polyps. If left untreated, all affected individuals will develop CRC. Mean age of colon cancer diagnosis in untreated individuals is 39 years. FAP accounts for about 1% of CRC and may also be associated with osteomas of the jaw, skull, and limbs; sebaceous cysts; and pigmented spots on the retina referred to as congenital hypertrophy of the retinal pigment epithelium. FAP associated with these collective extra-intestinal manifestations is sometimes referred to as Gardner syndrome. FAP may also be related to central nervous system tumors, referred to as Turcot syndrome.

Germline variants in the adenomatous polyposis coli (APC) gene, located on chromosome 5, are responsible for FAP and are inherited in an autosomal dominant manner. Variants in the APC gene result in altered protein length in about 80% to 85% of cases of FAP. A specific APC gene variant (I1307K) has been found in Ashkenazi Jewish descendants, which may explain a portion of the familial CRC occurring in this population.

A subset of FAP patients may have an attenuated form of FAP, typically characterized by fewer than 100 cumulative colorectal adenomas occurring later in life than in classical FAP. In the attenuated form of FAP, CRC occurs later in life (at an average age of 50 to 55 years) but lifetime risk of CRC remains high ($\approx 70\%$ by age 80 years). The risk of extra-intestinal cancer is also lower but cumulative lifetime risk remains high ($\approx 38\%$) compared with the general population.¹ Only 30% or fewer of attenuated FAP patients have APC variants; some of these patients have variants in the MUTYH (formerly MYH) gene, and this form of the condition is called MUTYH-associated polyposis (MAP). MAP occurs with a frequency similar to FAP, with some variability among prevalence estimates for both. While clinical features of MAP are similar to FAP or attenuated FAP, a strong multigenerational family history of polyposis is absent. Biallelic MUTYH variants are associated with a cumulative CRC risk of about 80% by age 70, whereas the monoallelic MUTYH variant-associated risk of CRC appears to be relatively minimal, although still under debate.² Thus, inheritance for high-risk CRC predisposition is autosomal recessive in contrast to FAP. When relatively few (i.e., between 10 and 99) adenomas are present, and family history is unavailable, the differential diagnosis may include both MAP and Lynch syndrome; genetic testing in this situation could include APC, MUTYH if APC is negative for variants, and screening for variants associated with Lynch syndrome.

It is important to distinguish among classical FAP, attenuated FAP, and MAP (mono- or biallelic) by genetic analysis because recommendations for patient surveillance and cancer prevention vary by syndrome.³

Testing

Genetic testing for APC variants may be considered in the following situations:

- Patients at high risk such as those with a family member who tested positive for FAP and have a known APC variant.
- Patients undergoing differential diagnosis of attenuated FAP versus MAP versus Lynch syndrome. These patients do not meet the clinical diagnostic criteria for classical FAP and have few adenomatous colonic polyps.
- To confirm FAP in patients with colon cancer with a clinical picture or family history consistent with classical FAP.

Lynch Syndrome

Lynch syndrome is an inherited disorder that results in a higher predisposition to CRC and other malignancies including endometrial and gastric cancer. Lynch syndrome is estimated to account for 3% to 5% of all CRC. People with Lynch syndrome have a 70% to 80% lifetime risk of developing any type of cancer.^{4,5} However the risk varies by genotype. It occurs as a result of germline variant in the mismatch repair (MMR) genes that include MLH1, MSH2, MSH6, and PMS2. In approximately 80% of cases, the variants are located in the MLH1 and MSH2 genes, while 10% to 12% of variants are located in the MSH6 gene and 2% to 3% in the PMS2 gene. Additionally, variants in three additional genes (MLH3, PMS1, EXO1) have been implicated with Lynch Syndrome. Notably, in individuals meeting the various clinical criteria for Lynch syndrome, 50% of individuals have a variant in the MLH1, MSH2, MSH6, and PMS2 genes. The lifetime risk of CRC is nearly 80% in individuals carrying a variant in one of these genes.

Testing

The testing approach to identify patients with Lynch syndrome is summarized next. Preliminary screening of

tumor tissue does not identify MMR gene variants but is used to guide subsequent diagnostic testing via DNA analysis for specific variants. Genetic testing or DNA analysis (gene sequencing, deletion and duplication testing) for the MMR genes involves assessment for MLH1, MSH2, MSH6, and PMS2 variants. The following are three testing strategies.

1. Microsatellite instability (MSI) testing (phenotype): Individuals with high MSI either proceed to genetic testing for MLH1, MSH2, MSH6, and PMS2 or to immunohistochemical (IHC) testing.
2. IHC testing (phenotype): Individuals with negative staining would proceed to genetic testing for MLH1, MSH2, MSH6, and PMS2.
3. Modification strategy: Tumor tissue of patients with negative staining for MLH1 on IHC is tested for the BRAF V600E variant to determine methylation status. If the BRAF variant is not detected, the individual receives MLH1 DNA analysis.

The phenotype tests used to identify individuals who may be at a high-risk of Lynch syndrome are explained next. The first screening test measures MSI. As a result of variance in the MMR gene family, the MMR protein is either absent or deficient, resulting in an inability to correct DNA replication errors causing MSI. Approximately 80% to 90% of Lynch syndrome CRC tumors have MSI. The National Cancer Institute has recommended screening for markers to detect MSI (Bethesda markers). MSI detection in two of these markers is considered a positive result or “high probability of MSI”.⁶

The second phenotype screening test is IHC, which involves the staining of tumor tissue for the presence of four MMR proteins (MLH1, MSH2, MSH6, PMS2). The absence of one or more of these proteins is considered abnormal.

BRAF testing is an optional screening method that may be used in conjunction with IHC testing for MLH1 to improve efficiency. A methylation analysis of the MLH1 gene can largely substitute for BRAF testing, or be used in combination to improve efficiency slightly.

Both MSI and IHC have a 5% to 10% false-negative rate. MSI testing performance depends on the specific MMR variant. MSI screening has a sensitivity of about 89% for MLH1 and MSH2 and 77% for MSH6 and a specificity of about 90% for each. The specificity of MSI testing is low because approximately 10% of sporadic CRCs are MSI-positive due to somatic hypermethylation of the MLH1 promoter. Additionally, some tumors positive for MSH6 variants are associated with the MSI-low phenotype rather than MSI-high; thus MSI-low should not be a criterion against proceeding to MMR variant testing.^{7,8} IHC screening has sensitivity for MLH1, MSH2, and MSH6 of about 83% and a specificity of about 90% for each.

Screening of tumor tissue from patients enables genetic testing for a definitive diagnosis of Lynch syndrome and leads to counseling, cancer surveillance (e.g., through frequent colonoscopic or endometrial screening examinations), and prophylaxis (e.g., risk-reducing colorectal or gynecologic surgeries) for CRC patients, as well as for their family members.

Genetic testing for an MMR gene variant is often limited to MLH1 and MSH2 and, if negative, then MSH6 and PMS2. The BRAF gene is often mutated in CRC when a particular BRAF variant (V600E, a change from valine to glutamic acid at amino acid position 600 in the BRAF protein) is present; to date, no MLH1 gene variants have been reported.⁹ Therefore, patients negative for MLH1 protein expression by IHC, and therefore potentially positive for an MLH1 variant, could first be screened for a BRAF variant. BRAF-positive samples need not be further tested by MLH1 sequencing. MLH1 gene methylation largely correlates with the presence of BRAF V600E and in combination with BRAF testing can accurately separate Lynch from sporadic CRC in IHC MLH1-negative cases.¹⁰

Recently, novel deletions have been reported to affect the expression of the MSH2 gene in the absence of an MSH2 gene variant, and thereby cause Lynch syndrome. In these cases, deletions in EPCAM, the gene for the

epithelial cell adhesion molecule, are responsible. EPCAM testing has been added to many Lynch syndrome profiles and is conducted only when tumor tissue screening results are MSI-high and/or IHC shows a lack of MSH2 expression, but no MSH2 variant is found by sequencing. EPCAM is found just upstream, in a transcriptional sense, of MSH2. Deletions of EPCAM that encompass the last two exons of the EPCAM gene, including the polyadenylation signal that normally ends transcription of DNA into messenger RNA, results in transcriptional “read-through” and subsequent hypermethylation of the nearby and downstream MSH2 promoter. This hypermethylation prevents normal MSH2 protein expression and leads to Lynch syndrome in a fashion similar to Lynch cases in which an MSH2 variant prevents MSH2 gene expression. Several studies have characterized such EPCAM deletions, established their correlation with the presence of EPCAM-MSH2 fusion messenger RNAs (apparently non-functional) and with the presence of MSH2 promoter hypermethylation, and, most importantly, have shown the cosegregation of these EPCAM variants with Lynch-like disease in families.¹¹⁻¹⁶

Distinct from patients with EPCAM deletions, rare cases of Lynch syndrome have been reported without detectable germline MMR variants, although IHC testing demonstrated a loss of expression of one of the MMR proteins. In at least some of these cases, research has identified germline “epivariants,” i.e., methylation of promoter regions that control the expression of the MMR genes.^{11,17,18} Such methylation may be isolated or be in conjunction with a linked genetic alteration near the affected MMR gene. The germline epivariants may arise de novo or may be heritable in Mendelian or non-Mendelian fashion. This is distinct from some cases of MSI-high sporadic CRC wherein the tumor tissue may show MLH1 promoter methylation and IHC nonexpression, but the same is not true of germline cells. Clinical testing for Lynch syndrome–related germline epivariants is not routine but may help in exceptional cases.

Female patients with Lynch syndrome have a predisposition to endometrial cancer. Lynch syndrome is estimated to account for 2% of all endometrial cancers in women and 10% of endometrial cancers in women younger than 50 years of age. Female carriers of the germline variants MLH1, MSH2, MSH6, and PMS2 have an estimated 40% to 62% lifetime risk of developing endometrial cancer, as well as a 4% to 12% lifetime risk of ovarian cancer.

Population Selection

Various attempts have been made to identify which patients with colon cancer should undergo testing for MMR variants, based primarily on family history and related characteristics using criteria such as the Amsterdam II criteria¹⁹ (low sensitivity but high specificity), Revised Bethesda guidelines²⁰ (better sensitivity but poorer specificity), and risk prediction models (e.g., MMRpro; PREMM5; MMRpredict).²¹ While family history is an important risk factor and should not be discounted in counseling families, it has poor sensitivity and specificity for identifying Lynch syndrome. Based on this and other evidence, the Evaluation of Genomic Applications in Practice and Prevention Working Group recommended testing all newly diagnosed CRC patients for Lynch syndrome, using a screening strategy based on MSI or IHC (with or without BRAF) followed by sequencing in screen-positive patients. This recommendation includes genetic testing for the following types of patients:

- Family members of Lynch syndrome patients with a known MMR variant; family members would be tested only for the family variant; those testing positive would benefit from early and increased surveillance to prevent future CRC.
- Patients with a differential diagnosis of Lynch syndrome versus attenuated FAP vs. MAP.
- For Lynch syndrome patients, genetic testing of the proband with CRC likely benefits the proband where Lynch syndrome is identified, and appropriate surveillance for associated malignancies can be initiated and maintained and benefits family members by identifying the family variant.

Juvenile Polyposis Syndrome

JPS is an autosomal dominant genetic disorder characterized by the presence of multiple hamartomatous (benign) polyps in the digestive tract. It is rare, with an estimated incidence of one in 100,000 to 160,000. Generalized juvenile polyposis refers to polyps in the upper and lower gastrointestinal tract, and juvenile polyposis coli refers to polyps of the colon and rectum. Those with JPS are at a higher risk for colorectal and gastric cancer.²² Approximately 60% of patients with JPS have a germline variant in the BMPR1A gene or the SMAD4 gene.^{23, 24} Approximately 25% of patients have de novo variants.^{25, 26} In most cases, polyps appear in the first decade of life and most patients are symptomatic by age 20 years.²⁷ Rectal bleeding is the most common presenting symptom, occurring in more than half of patients. Other presenting symptoms include prolapsing polyp, melena, pain, iron deficiency anemia, and diarrhea.^{22, 26, 27}

As noted, individuals with JPS are at increased risk for colorectal and gastric cancer. By 35 years of age, the cumulative risk of CRC is 17% to 22%, which increases to 68% by age 60 years.^{28, 29} The estimated lifetime risk of gastric cancer is 20% to 30%, with a mean age at diagnosis of 58 years.^{22, 26, 28} JPS may also be associated with hereditary hemorrhagic telangiectasia.³⁰ The most common clinical manifestations of hereditary hemorrhagic telangiectasia are telangiectasias of the skin and buccal mucosa, epistaxis, and iron deficiency anemia from bleeding.

Diagnosis

A clinical diagnosis of JPS is made on the basis of the presence of any one of the following: at least three to five juvenile polyps in the colon or multiple juvenile polyps in other parts of the gastrointestinal tract or any number of juvenile polyps in a person with a known family history of juvenile polyps.³¹ It is recommended that individuals who meet clinical criteria for JPS undergo genetic testing for a germline variant in the BMPR1A and SMAD4 genes for a confirmatory diagnosis of JPS and to counsel at-risk family members. If there is a known SMAD4 variant in the family, genetic testing should be performed within the first six months of life due to hereditary hemorrhagic telangiectasia risk.³¹

Peutz-Jeghers Syndrome

PJS is also an autosomal dominant genetic disorder, similar to JPS, and characterized by the presence of multiple hamartomatous (benign) polyps in the digestive tract, mucocutaneous pigmentation, and an increased risk of gastrointestinal and nongastrointestinal cancers. It is rare, with an estimated incidence of one in 8000 to 200,000. In most cases, a germline variant in the STK11 (LKB1) gene is responsible for PJS, which has a high penetrance of over 90% by the age of 30 years.³²⁻³⁴ However, 10% to 20% of individuals with PJS have no family history and are presumed to have PJS due to de novo variants.³⁵ A variant in STK11 is detected in only 50% to 80% of families with PJS, suggesting that there is a second PJS gene locus.

The reported lifetime risk for any cancer is between 37% and 93% among those diagnosed with PJS with an average age of cancer diagnosis at 42 years. The most common sites for malignancy are colon and rectum, followed by breast, stomach, small bowel, and pancreas.³⁶ The estimated lifetime risk of gastrointestinal cancer ranges from 38% to 66%.³⁶ Lifetime cancer risk stratified by organ site is colon and rectum (39%), stomach (29%), small bowel (13%), and pancreas (11%-36%).

Diagnosis

A clinical diagnosis of PJS is made if an individual meets two or more of the following criteria: the presence of two or more histologically confirmed PJ polyps of the small intestine or characteristic mucocutaneous pigmentation of the mouth, lips, nose, eyes, genitalia, fingers, or family history of PJS.³¹ Individuals who meet clinical criteria for PJS should undergo genetic testing for a germline variant in the STK11 gene for a confirmatory diagnosis of PJS and counseling at-risk family members

REGULATORY STATUS

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests (LDTs) must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). Genetic tests reviewed in this protocol are available under the auspices of CLIA. Laboratories that offer LDTs must be licensed by CLIA for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of this test.

RELATED PROTOCOL

KRAS, NRAS, and BRAF Variant Analysis in Metastatic Colorectal Cancer

Services that are the subject of a clinical trial do not meet our Technology Assessment Protocol criteria and are considered investigational. *For explanation of experimental and investigational, please refer to the Technology Assessment Protocol.*

It is expected that only appropriate and medically necessary services will be rendered. We reserve the right to conduct prepayment and postpayment reviews to assess the medical appropriateness of the above-referenced procedures. **Some of this protocol may not pertain to the patients you provide care to, as it may relate to products that are not available in your geographic area.**

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