The following Protocol contains medical necessity criteria that apply for this service. It is applicable to Medicare Advantage products unless separate Medicare Advantage criteria are indicated. If the criteria are not met, reimbursement will be denied and the patient cannot be billed. Preauthorization is required.* 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.

Description

Genetic testing is available for both affected individuals, as well as those at risk, for various types of hereditary colon cancer. This Protocol describes genetic testing for familial adenomatous polyposis (FAP), Lynch syndrome (formerly known as HNPCC), as well as MYH-associated polyposis.

There are currently two well-defined types of hereditary colorectal cancer, familial adenomatous polyposis (FAP) and Lynch syndrome (formerly, hereditary nonpolyposis colorectal cancer or HNPCC).

Familial adenomatous polyposis 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 go on to develop colorectal cancer. The mean age of colon cancer diagnosis in untreated individuals is 39 years. FAP accounts for about 1% of colorectal cancer 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 (CHRPE). FAP associated with these collective extraintestinal manifestations is sometimes referred to as Gardner syndrome. FAP may also be associated with CNS tumors, referred to as Turcot syndrome.

Germline mutations in the adenomatous polyposis coli (APC) gene, located on chromosome 5, are responsible for FAP and are inherited in an autosomal dominant manner. Mutations in the APC gene result in altered protein length in about 80% to 85% of cases of FAP. A specific APC gene mutation (I1307K) has been found in subjects of Ashkenazi Jewish descent that may explain a portion of the familial colorectal cancer occurring in this population.

A subset of FAP patients may have attenuated FAP (AFAP), typically characterized by fewer than 100 cumulative colorectal adenomas occurring later in life than in classical FAP, colorectal cancer occurring at an average age of 50-55 years, but a high lifetime risk of colorectal cancer of about 70% by age 80. The risk of extra-intestinal cancer is lower compared to classical FAP, but still high at an estimated cumulative lifetime risk of 38% compared to the general population. (1) Only 30% or fewer of AFAP patients have APC mutations; some of these patients instead have mutations in the MUTYH (formerly MYH) gene and are then diagnosed with MUTYH-associated polyposis (MAP). MAP occurs with a frequency approximately equal to FAP, with some variability among prevalence estimates for both. While clinical features of MAP are similar to FAP or AFAP, a strong multigenerational family history of polyposis is absent. Biallelic MUTYH mutations are associated with a cumulative colorectal cancer risk of about 80% by age 70, whereas monoallelic MUTYH mutation-associated risk of colorectal cancer appears to be relatively minimal, although still under debate. (2) Thus, inheritance for high-risk colorectal cancer 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 mutations, and screening for mutations 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 according to the syndrome. (3)

Genetic testing for APC mutations may be considered for the following types of patients:

- Family members of patients with FAP and a known APC mutation. Those without the specific mutation have not inherited the susceptibility gene and can forego intense surveillance (although they retain the same risk as the general population and should continue an appropriate level of surveillance).
- Patients with a differential diagnosis of attenuated FAP vs. MUTYH-associated polyposis vs. Lynch syndrome. These patients do not meet the clinical diagnostic criteria for classical FAP, and have few adenomatous colonic polyps.
- Patients with colon cancer with a clinical picture or family history consistent with classical FAP.

Lynch syndrome

Patients with Lynch syndrome have a predisposition to colorectal cancer and other malignancies as a result of an inherited mutation in a DNA mismatch repair (MMR) gene. Lynch syndrome includes those with an existing cancer and those who have not yet developed cancer. The term “hHNPCC” originated prior to the discovery of explanatory MMR mutations for many of these patients, and now includes some who are negative for MMR mutations and likely have mutations in as-yet unidentified genes. For purposes of clarity and analysis, the use of Lynch syndrome in place of HNPCC has been recommended in several recent editorials and publications.

Lynch syndrome is estimated to account for 3% to 5% of all colorectal cancer and is also associated with an increased risk of other cancers such as endometrial, ovarian, urinary tract, and biliary tract cancer. Lynch syndrome is associated with a risk of developing colorectal cancer by age 70 of approximately 27% to 45% for men, and 22% to 38% for women, after correction for ascertainment bias. (4) Lynch syndrome patients who have colorectal cancer also have an estimated 16% risk of a second primary within 10 years.

Lynch syndrome is associated with any of a large number of possible mutations in one of several MMR genes, known as MLH1, MSH2, MSH6, PMS2 and rarely MLH3. Risk of all Lynch syndrome-related cancers is markedly lower for carriers of a mutation in the MSH6 and PMS2 genes, although for most cancers still significantly higher than that of the general population. (3, 4) Estimated cumulative risks of any associated cancer for a carrier of a mutation in any MMR gene do not begin to increase until after age 30.

Lynch syndrome mutations are heterozygous; that is, only one of the two gene alleles contains a mutation. In rare cases both alleles contain the mutation, i.e., biallelic MMR gene mutations. This unusual syndrome has been described in multiple families and is to a large extent the result of consanguinity. (5) Children with biallelic MMR mutations may develop extracolonic cancers in childhood, such as brain tumors, leukemias, or lymphomas. Those unaffected or surviving early malignancies are at high risk of later colorectal cancer (average age of colorectal cancer diagnosis 16.4 years (5)). Family history may not suggest Lynch syndrome. Prior to cancer diagnosis, patients may have multiple adenomatous polyps, and thus may have an initial differential diagnosis of attenuated FAP versus MUTYH-associated polyposis versus Lynch syndrome.

About 70% of Lynch syndrome patients have mutations in either MLH1 or MSH2. Testing for MMR gene mutations is often limited to MLH1 and MSH2 and, if negative, then MSH6 and PMS2 testing. Large gene sizes and the difficulty of detecting mutations in these genes make direct sequencing a time- and cost-consuming process. Thus, additional indirect screening methods are needed to determine which patients should proceed to
direct sequencing for MMR gene mutations. Available screening methods are microsatellite instability (MSI) testing or immunohistochemical (IHC) testing. 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 slightly improve efficiency.

Mutations in MMR genes result in a failure of the mismatch repair system to repair errors that occur during the replication of DNA in tumor tissue. Such errors are characterized by the accumulation of alterations in the length of simple, repetitive microsatellite (two to five base repeats) sequences that are distributed throughout the genome, termed microsatellite instability (MSI) and resulting in a MSI-high tumor phenotype. MSI testing was standardized subsequent to a 2004 National Cancer Institute (NCI) workshop. Methodologic studies have also shown the importance of laser microdissection of the tumor tissue, comparison of tumor and normal cells, and a minimum proportion of tumor in relation to the quality of the test results. While the sensitivity of MSI testing is high, the specificity is low because approximately 10% of sporadic CRC are MSI-positive due to somatic hypermethylation of the MLH1 promoter. Additionally, some tumors positive for MSH6 mutations are associated with the MSI-low phenotype, rather than MSI-high; thus MSI-low should not be a criterion against proceeding to MMR mutation testing. (7, 8)

Absent or reduced protein expression may be a consequence of an MMR gene mutation. IHC assays for the expression of MLH1, MSH2, MSH6, and PMS2 can be used to detect loss of expression of these genes and to focus sequencing efforts on a single gene. It is also possible for IHC assays to show loss of expression, and thus indicate the presence of a mutation, when sequencing is negative for a mutation. In such cases, mutations may be in unknown regulatory elements and cannot be detected by sequencing of the protein coding regions. Thus IHC may add additional information.

The BRAF gene is often mutated in colorectal cancer; when a particular BRAF mutation (V600E, a change from valine to glutamic acid at amino acid position 600 in the BRAF protein) is present; to date no MLH1 gene mutations have been reported. Therefore, patients negative for MLH1 protein expression by IHC, and therefore potentially positive for an MLH1 mutation, could first be screened for a BRAF mutation. 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 colorectal cancer in IHC MLH1-negative cases. (10)

Various attempts have been made to identify which patients with colon cancer should undergo testing for MMR mutations, based primarily on family history and related characteristics using criteria such as the Amsterdam II criteria (low sensitivity but high specificity) and the Bethesda guidelines (better sensitivity but poorer specificity). 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 (EGAPP) Working Group recommended testing all newly diagnosed patients with colorectal cancer for Lynch syndrome, using a screening strategy based on MSI or IHC (+ 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 mutation; family members would be tested only for the family mutation; those testing positive would benefit from early and increased surveillance to prevent future colorectal cancer.
- Patients with a differential diagnosis of Lynch syndrome vs. attenuated FAP vs. MYH-associated polyposis.
- Lynch syndrome patients. Genetic testing of the proband with colorectal cancer 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 mutation.
Recently, novel deletions have been reported to affect the expression of the \textit{MSH2} MMR gene in the absence of a \textit{MSH2} gene mutation, and thereby cause Lynch syndrome. In these cases, deletions in \textit{EPCAM}, the gene for the epithelial cell adhesion molecule are responsible. \textit{EPCAM} testing has been added to many Lynch syndrome profiles and is conducted only when tumor tissue screening results are MSI-high, and IHC shows a lack of MSH2 expression, but no MSH2 mutation is found by sequencing.

Separately from patients with EPCAM deletions, rare Lynch syndrome patients have been reported without detectable germline MMR mutations although IHC testing demonstrates a loss of expression of one of the MMR proteins. In at least some of these cases, research has identified germline ‘epimutations,’ i.e., methylation of promoter regions that control the expression of the MMR genes. (11-13) Such methylation may be isolated, or in conjunction with a linked genetic alteration near the affected MMR gene. The germline epimutations may arise de novo, or may be heritable in either Mendelian or non-Mendelian fashion. This is distinct from some cases of MSI-high sporadic colorectal cancer wherein the tumor tissue may show MLH1 promotor methylation and IHC non-expression, but the same is not true of germline cells. Clinical testing for Lynch syndrome-related germline epimutations is not routine but may be helpful in exceptional cases. Epimutations as a cause of Lynch syndrome are described only for informational purposes; no policy statement is made regarding this testing.

**Corporate Medical Guideline**

Genetic testing for APC gene mutations may be considered \textbf{medically necessary} in the following patients:

- At-risk relatives (see Policy Guidelines) of patients with FAP and/or a known APC mutation.
- Patients with a differential diagnosis of attenuated FAP vs. \textit{MUTYH}-associated polyposis vs. Lynch syndrome. Whether testing begins with APC mutations or screening for MMR mutations depends upon clinical presentation.

Genetic testing for APC gene mutations is \textbf{not medically necessary} for colorectal cancer patients with classical FAP for confirmation of the FAP diagnosis.

Genetic testing for \textit{MUTYH} gene mutations may be considered \textbf{medically necessary} in the following patients:

- Patients with a differential diagnosis of attenuated FAP vs. \textit{MUTYH}-associated polyposis vs. Lynch syndrome and a negative result for APC gene mutations. Family history of no parents or children with FAP is consistent with \textit{MUTYH}-associated polyposis (autosomal recessive).

Genetic testing for MMR gene mutations is considered \textbf{medically necessary} in the following patients:

- Patients with colorectal cancer, for the diagnosis of Lynch syndrome (see Policy Guidelines Section).
- At-risk relatives (see Policy Guidelines) of patients with Lynch syndrome with a known MMR mutation.
- Patients with a differential diagnosis of attenuated FAP vs. \textit{MUTYH}-associated polyposis vs. Lynch syndrome. Whether testing begins with APC mutations or screening for MMR mutations depends upon clinical presentation.
- Patients without colorectal cancer but with a family history meeting the Amsterdam or Revised Bethesda criteria, when no affected family members have been tested for MMR mutations.

Genetic testing for EPCAM mutations is considered \textbf{medically necessary} when any one of the following three major criteria is met:

- Patients with colorectal cancer, for the diagnosis of Lynch syndrome (see Policy guidelines Section) when:
  - a. Tumor tissue shows lack of \textit{MSH2} expression by immunohistochemistry and patient is negative for a germline mutation in \textit{MSH2}; OR
b. Tumor tissue shows a high level of microsatellite instability and patient is negative for a germline mutation in MSH2, MLH1, PMS2, and MSH6; OR

2. At-risk relatives (see Policy Guidelines) of patients with Lynch syndrome with a known EPCAM mutation; OR

3. Patients without colorectal cancer but with a family history meeting the Amsterdam or Revised Bethesda criteria, when no affected family members have been tested for MMR mutations, and when sequencing for MMR mutations is negative.

Pre- and post-test genetic counseling may be considered medically necessary as an adjunct to the genetic testing itself.

Policy Guideline

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

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 mutation found in the affected family member.

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

For patients with colorectal cancer being evaluated for Lynch syndrome, either the microsatellite instability (MSI) test, or the immunohistochemistry (IHC) test with or without BRAF gene mutation testing, should be used as an initial evaluation of tumor tissue prior to MMR gene analysis. Both tests are not necessary. Consideration of proceeding to MMR gene sequencing would depend on results of MSI or IHC testing. IHC testing in particular may help direct which MMR gene likely contains a mutation, if any, and may also provide some additional information if MMR genetic testing is inconclusive.

When indicated, genetic sequencing for MMR gene mutations 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 mutations are expected based on IHC or MSI studies but none are found by standard sequencing, additional testing for large deletions or duplications is appropriate.

The COLARIS test from Myriad Genetic Laboratories includes sequence analysis of MLH1, MSH2, MSH6 and PMS2; large rearrangement analysis for MLH1 MSH2, PMS2, and MSH6 large deletions/duplications; and analysis for large deletions in the EPCAM gene near MSH2. Note that there may be two versions of this test, the COLARIS (excludes PMS2 testing) and COLARIS Update (includes PMS2 testing). Testing is likely done in stages beginning with the most common types of mutations. Individualized tested (e.g., targeted testing for a family mutation) can also be requested.

The COLARIS® AP test from Myriad Genetic Laboratories includes DNA sequencing analysis of the APC and MUTYH genes as well as analysis of large rearrangements in the APC gene that are not detected by DNA sequencing.

Amsterdam II Clinical Criteria (all criteria must be fulfilled) (14). The Amsterdam criteria are the most stringent criteria for defining families at high risk for Lynch Syndrome:
• Three or more relatives with an associated cancer (colorectal cancer, 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 (FAP) should be excluded in cases of colorectal carcinoma;
• Tumors should be verified by pathologic examination.
• Modifications:
  ▪ EITHER: very small families, which cannot be further expanded, can be considered to have HNPCC with only two colorectal cancers in first-degree relatives if at least two generations have the cancer and at least one case of colorectal cancer was diagnosed by the age of 55 years;
  ▪ OR: in families with two first-degree relatives affected by colorectal cancer, the presence of a third relative with an unusual early-onset neoplasm or endometrial cancer is sufficient.

Revised Bethesda Guidelines (fulfillment of any criterion meets guidelines) (6). The Bethesda guidelines are less strict than the Amsterdam criteria and are intended to increase the sensitivity of identifying at-risk families. The Bethesda guidelines are also felt to be more useful in identifying which patients with colorectal cancer should have their tumors tested for microsatellite instability and/or immunohistochemistry:
• Colorectal carcinoma (CRC) diagnosed in a patient who is less than 50 years old;
• Presence of synchronous (at the same time) or metachronous (at another time i.e.- a recurrence of) CRC or other Lynch syndrome-associated tumors, regardless of age;
• CRC with high microsatellite instability histology diagnosed in a patient less 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 at less than 50 years of age;
• CRC diagnosed in two or more first-degree or second-degree relatives with Lynch syndrome-associated tumors, regardless of age. Lynch-associated tumors include: endometrial, stomach, ovarian, cervical, esophageal, leukemia, thyroid, bladder, ureter and renal pelvis, biliary tract, small bowel, breast, pancreas, liver, larynx, bronchus, lung, and brain (glioblastoma), sebaceous gland adenomas, and keratoacanthomas.

Genetic testing for colon cancer is not widely available and is most commonly performed by commercial reference labs or research labs dedicated to genetic testing in general.

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.
References

We are not responsible for the continuing viability of web site addresses that may be listed in any references below.


