Interpretation of Genetic Testing for Lynch Syndrome in Patients With Putative Familial Colorectal Cancer

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Key Words
Lynch, colorectal, genetic testing, HNPCC, mismatch repair

Abstract
Colorectal cancer (CRC) risk assessment involves the evaluation of an individual’s personal and family history for characteristics of an inherited susceptibility to develop CRC. Lynch syndrome (LS), or hereditary nonpolyposis colorectal cancer, is the most common cause of hereditary CRC, underlying 2% to 3% of patients with newly diagnosed (incident) CRC. Risk assessment for LS is complex, and the interpretation of the many available tests can be challenging even for the genetics specialist. A move toward universal (reflex) LS screening for mismatch repair in all patients with incident CRC supports the importance of improving the awareness and understanding of LS testing, teaching rational testing approaches, and honing interpretive skills among cancer care providers. This article reviews important clinical features of LS genetic evaluation using 3 pedigree-based case examples from the Fox Chase Cancer Center Gastrointestinal Risk Assessment Clinic. (JNCCN 2011;9:1311–1320)

Colorectal cancer (CRC) risk assessment involves evaluation of an individual’s personal and family history for characteristics of an inherited susceptibility to develop CRC. These characteristics include early age at diagnosis, multiple primary cancers, polyposis, family history of syndrome-specific malignancies, and certain histologic features. In the absence of polyposis, the genetic testing algorithm for hereditary CRC focuses largely on identifying Lynch syndrome (LS), also known as hereditary nonpolyposis colorectal cancer. Previously, an understanding of the workup of individuals suspected of having LS could be left to those specializing in genetics. Recently, screening of all patients with newly diagnosed CRC for LS independent of family history has been endorsed by the CDC-sponsored Evaluation of Genomic Applications in Practice and Prevention Working Group (EGAPP) and others. However, although highlighting the EGAPP recommendations, the current NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) for Colorectal Cancer Screening continue to endorse mismatch repair (MMR) screening in individuals with a strong family history for colon cancer (to view the most recent version of these guidelines, visit the NCCN Website at www.NCCN.org).1–3 Several centers, including Fox Chase Cancer Center (FCCC), are implementing universal screening programs. A need is emerging for clinicians to be able to converse with patients about the value of tests to evaluate hereditary CRC risk, and to facilitate the interpretation of the results.

Updates to the understanding of the pathogenesis of LS present an enormous challenge for clinicians attempting to remain knowledgeable about hereditary cancer syndromes and seeking to provide ongoing comprehensive genetic risk information to current and past patients. Although tumor testing and risk evaluation have become integral aspects of medical oncology care, the complete process of cancer genetic counseling, from the construction of a 3-generation pedigree to the interpretation of results, is a complex task that may require more time and resources than many physicians are able to provide. The cancer genetics specialist is expert at choosing the appropriate test, with the ability to integrate new information based on updates in testing technique, the identification of new genes, and increased understanding of the variable presentation of susceptibility...
conditions. Cancer genetics specialists can be found through the National Society of Genetic Counselors (www.nsgc.org) and the National Cancer Institute (www.cancer.gov or 1–800–4–CANCER).

Lynch Syndrome Etiology and Identification

LS is the most common cause of hereditary CRC, accounting for 2.7% of all CRC cases\(^6,7\) and 2.1% of all endometrial cancer cases.\(^6,7\) The prevalence of LS in the general population is estimated to be 1 in 300.\(^1\) LS is associated with increased risks for multiple malignancies in addition to CRC and endometrial cancer, including cancer of the stomach, ovary, renal pelvis, ureter, biliary tract, small bowel, brain, and sebaceous glands.\(^8,9\) LS is caused by mutations in the MMR genes MLH1, MSH2, MSH6, and PMS2,\(^10–13\) and by deletions in the EPCAM gene.\(^14–16\) The characteristic phenotype of LS tumors is microsatellite instability (MSI), a sign of defective MMR.\(^17,18\) Microsatellites are areas of the genome at which the DNA sequence is repetitive (e.g., (CA)\(_n\)). During DNA replication, errors may occur that are usually corrected by the MMR protein complex, including the MLH1, MSH2, MSH6, and PMS2 proteins.\(^19,20\) Individuals with deficient MMR (dMMR), such as those with LS, will have difficulty repairing mismatches and their tumors will exhibit expansions and contractions in the microsatellite regions. These tumors will also show loss of immunohistochemical expression of one or more of the MMR proteins.\(^21\) MMR testing using MSI and immunohistochemical expression can be performed on formalin-fixed, paraffin-embedded tissue. An advantage of immunohistochemical testing is that it can be performed in the pathology department of most hospitals, whereas MSI testing requires molecular facilities. Based on a recent extensive evidence review by the EGAPP, the sensitivity of MSI testing is estimated to be 69% to 85% and the sensitivity of immunohistochemical testing is 83% in LS CRCs.\(^22\) Nonetheless, research has also highlighted several pitfalls of routine immunohistochemical testing for the LS proteins, including issues related to quality control and interobserver variability in interpretation.\(^23,24\) The dMMR phenotype is also present in LS-associated endometrial cancers at a high frequency.\(^25\)

Sporadic Mismatch Repair Deficiency

Approximately 15% of all CRCs will exhibit the dMMR\(^17,18,26\) phenotype, yet only a portion (approximately 3%) are from LS.\(^4,5,27\) The remaining 12% will exhibit MSI and show loss of MLH1 and PMS2 on immunohistochemical analysis. In these cases, dMMR is caused by acquired hypermethylation of the MLH1 gene promoter.\(^28,29\) This feature is a somatic change associated with advanced age, female sex, and location (with the proximal colon affected more often than the distal).\(^30,31\) The addition of methyl groups in the MLH1 promoter blocks transcription factor binding and effectively silences the MLH1 gene in colonic epithelial cells.\(^29,32\) Because the MLH1 and PMS2 proteins are heterodimers, PMS2 will generally also degrade if the MLH1 gene is not transcribed, resulting in loss of both MLH1 and PMS2 on clinical immunohistochemical staining.\(^20\)

Tumor-based molecular testing for the common Braf V600E mutation has been shown to be useful in distinguishing sporadic LS-associated dMMR. Braf encodes a kinase involved in response to growth signals. Braf mutation leads to constitutive activity of the kinase.\(^33\) Braf V600E accounts for approximately 90% of all activating Braf mutations.\(^3,31\) Clinically, most CRCs with dMMR from acquired hypermethylation of MLH1 (40%–83%) are characterized by the Braf V600E mutation, whereas this mutation has not been seen in tumors from germline MLH1 loss.\(^34–36\) Although at least one case has been reported.\(^35\) Thus, the identification of Braf V600E in a CRC tumor exhibiting MSI supports that further evaluation for germline MLH1 mutations is unwarranted.

Clinical Criteria for LS

The identification of individuals with LS has been a consistent challenge, owing largely to its variable presentation and the difficulty of obtaining an accurate and comprehensive family history. Many clinical definitions and testing algorithms for LS have been proposed over time. Before the elucidation of the molecular basis of LS, the Amsterdam criteria I were developed to provide criteria for recruiting patients into collaborative studies. These criteria describe families 1) that have had 3 cases of CRC, with 2 of the affected individuals being first-degree relatives of the third; 2) with CRCs occurring in at least 2 gen-
The Bethesda guidelines were originally developed to guide tumor-based MSI screening for LS in patients with CRC and were updated to their current form (the revised Bethesda guidelines) in 2003.\(^\text{40,41}\) The Bethesda guidelines consider personal and family history in addition to histologic features more common in high-level MSI (MSI-H) tumors, such as Crohn’s disease–like lymphocytic reaction, the presence of tumor-infiltrating lymphocytes, and mucinous and signet ring appearance.\(^\text{40}\) These modifications make the Bethesda guidelines more sensitive than the Amsterdam criteria (94% vs. 61%) for detecting individuals with germline LS mutation, but at the cost of loss of specificity (25% vs. 67%).\(^\text{42}\)

### Risk Assessment for Lynch Syndrome

At FCCC, the approach to the evaluation of possible LS in a family has been to begin with MSI and immunohistochemical testing on the tumor of the family member with the youngest age at diagnosis of CRC or endometrial cancer. The advantage of beginning with MSI and immunohistochemical testing in tandem is the sensitivity of tumor-based tests for detecting the MMR phenotype,\(^\text{5,27,43}\) which helps to establish a clinical diagnosis of LS in families with suggestive histories.

A proficient MMR (pMMR) phenotype from tumor-based testing in an appropriate family member is extremely reassuring (i.e., highly sensitive) that LS is not the underlying cause of that tumor, although phenocopy cancers (i.e., a sporadic case of cancer within a putative LS family) must always be considered. Molecular genetic testing will miss approximately 15% of individuals with clinical LS. Recent updates in testing technique and the discovery of new genes associated with LS have exposed the weakness of molecular LS testing for detecting mutation carriers. Even novel mechanisms of LS not owing to mutations in MMR genes, such as EPCAM deletions and constitutional MLHI epimutations, will result in the dMMR phenotype.\(^\text{15,16,44}\) In addition, immunohistochemical testing in particular can define MMR status and guide the genetic testing process, pinpointing the specific gene in which the mutation exists. For example, loss of MSH6 only on immunohistochemical testing indicates that genetic analysis of MSH6 should be pursued. This can save time and cost associated with pursuing testing in the 4 other LS genes. Table 1 summarizes the abnormal immunohistochemical testing results that are most frequently encountered in clinical practice and their interpretations. The NCCN Guidelines for Colorectal Cancer Screening\(^\text{1}\) provide a more detailed table that combines MSI, BRAF V600E, and methylation results (available online at www.NCCN.org [LS-A, page 2 of 2]).

Although acquiring permissions and tumor specimens from relatives of a proband can be challenging, tumor blocks are retained for at least 10 years and are generally adequate for testing. Tumor-based MMR screening several years after a CRC or endometrial cancer diagnosis may be associated with out-of-pocket costs to families if the proband is deceased, but these costs are balanced by the value of the information the screening provides. Particularly because germline DNA-based testing for LS in unaffected individuals remains uncovered by some insurance carriers, this approach can also help reduce patient out-of-pocket costs through focused testing as opposed to complete analysis of the 5 LS-associated genes, which may cost up to $8000 or more.

Recently, FCCC began transitioning to a universal (reflex) LS screening strategy (Figure 1). Supported by the EGAPP recommendations, and partially modeled based on the experience reported by Ohio State University, the FCCC reflex MMR screening strategy will use immunohistochemical testing as the primary screen for MMR in all new patients with surgically resected or biopsied CRC and endometrial cancer cases, with MSI performed only when a failed or uninterpretable immunohistochemical test is encountered. Individuals for whom immunohistochemistry shows a loss of PMS2, MSH2, and/or MSH6 will be directed to genetic counseling and germline DNA testing. When loss of MLH1/PMS2 is seen on immunohistochemistry, individuals with endometrial cancer will be directed for genetic counseling for consideration of methylation or germline testing. Those with CRC will have BRAF
V600E testing before being referred for counseling to identify sporadic cases. Those who exhibit dMMR on MSI will be referred for genetic counseling for consideration of BRAF V600E testing and/or 5-gene analysis. High-risk individuals referred for genetic counseling and risk assessment because of a suspicion of LS based on personal or family history will continue to have both MSI and immunohistochemical testing performed to assess for LS, because these tests combined provide very high sensitivity for detecting the dMMR phenotype. The authors believe both are warranted as part of a comprehensive LS genetics evaluation in high-risk patients.

Beyond its use in identifying LS, evaluation of MMR status may, in the near future, become an increasingly integral part of the prognostication and treatment planning for patients with newly diagnosed CRC. The superior prognosis of patients with dMMR tumors over those with pMMR has been established, and data strongly suggest that stage II colon tumors with dMMR are unlikely to benefit from 5-FU–based adjuvant chemotherapy.

**Difficulties in LS Evaluation: Clinical Cases**

Risk assessment for LS is complex, and difficulties in clinical decision-making are common. In the following cases, the authors illuminate several difficulties they have encountered in their clinical practice. Table 2 provides a referenced summary that includes these scenarios and several others that are not discussed in detail.

**Case 1**

**Personal History:** A 56-year-old man with recently diagnosed stage IIA CRC was referred for cancer risk assessment by his medical oncologist. The physician ordered MSI and immunohistochemical testing on the colon tumor specimen to inform use of adjuvant 5-FU–based chemotherapy. This testing showed MSI-H and loss of MLH1 and PMS2 expression on immunohistochemistry (Figure 2A).

**Family History:** The paternal history included a father with CRC (age, 60 years), an uncle with CRC (age, 64 years), and an aunt with gastric cancer.

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**Table 1 Interpreting Common Abnormal Results on Immunohistochemical Testing**

<table>
<thead>
<tr>
<th>Antibody/Protein</th>
<th>Result</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Result 1:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MLH1</td>
<td>Present staining/expression</td>
<td>ABNORMAL Highly suspicious for Lynch syndrome because of a PMS2 mutation.</td>
</tr>
<tr>
<td>MSH2</td>
<td>Present staining/expression</td>
<td></td>
</tr>
<tr>
<td>MSH6</td>
<td>Present staining/expression</td>
<td></td>
</tr>
<tr>
<td>PMS2</td>
<td>Absent staining/expression</td>
<td></td>
</tr>
<tr>
<td>Result 2:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MLH1</td>
<td>Present staining/expression</td>
<td>ABNORMAL Highly suspicious for Lynch syndrome because of an MSH6 mutation.</td>
</tr>
<tr>
<td>MSH2</td>
<td>Present staining/expression</td>
<td></td>
</tr>
<tr>
<td>MSH6</td>
<td>Absent staining/expression</td>
<td></td>
</tr>
<tr>
<td>PMS2</td>
<td>Present staining/expression</td>
<td></td>
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<tr>
<td>Result 3:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MLH1</td>
<td>Present staining/expression</td>
<td>ABNORMAL Highly suspicious for Lynch syndrome, likely because of an MSH2 mutation; other causes include EPCAM deletions and, rarely, MSH6 mutations.</td>
</tr>
<tr>
<td>MSH2</td>
<td>Absent staining/expression</td>
<td></td>
</tr>
<tr>
<td>MSH6</td>
<td>Absent staining/expression</td>
<td></td>
</tr>
<tr>
<td>PMS2</td>
<td>Present staining/expression</td>
<td></td>
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<tr>
<td>Result 4:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MLH1</td>
<td>Absent staining/expression</td>
<td>ABNORMAL May be caused by Lynch syndrome because of an MLH1 mutation or may be from acquired (somatic) MLH1 methylation. Germline testing may identify MLH1 mutation carriers. For individuals without a strong personal or family history, further tumor testing (methylation and/or BRAF testing) may be a more cost-effective option than germline testing.</td>
</tr>
<tr>
<td>MSH2</td>
<td>Present staining/expression</td>
<td></td>
</tr>
<tr>
<td>MSH6</td>
<td>Present staining/expression</td>
<td></td>
</tr>
<tr>
<td>PMS2</td>
<td>Absent staining/expression</td>
<td></td>
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</tbody>
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Testing Strategy: Germline DNA testing of the MLH1 gene showed no mutation. Tumor analysis for the BRAF V600E mutation was negative. However, because this test is not completely sensitive for hypermethylation of the MLH1 gene promoter, MLH1 methylation-specific polymerase chain reaction was subsequently performed. This test was positive and provided a basis for the abnormal dMMR result.

Discussion: The dMMR phenotype is not exclusive to LS, and in fact is more frequently from a non-hereditary cause. The 12% of cancers that have somatic methylation of the MLH1 promoter will have

Table 2 Challenges for Lynch Syndrome Risk Assessment

<table>
<thead>
<tr>
<th>Difficulty in Lynch Syndrome Evaluation</th>
<th>Clinical Consideration</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>All surgical CRC and EC tumors</td>
<td>Universal MMR screening</td>
<td></td>
</tr>
<tr>
<td>Genetic testing fails to detect some LS mutations.</td>
<td>MMR screening with IHC</td>
<td></td>
</tr>
<tr>
<td>Approximately 50% of families meeting AC-I (CRC only) will have no LS gene mutation.</td>
<td>IHC with MLH1/PMS2 loss</td>
<td></td>
</tr>
<tr>
<td>The sensitivity of MSI and IHC for non-CRC/EC LS cancers is unclear.</td>
<td>IHC with MSH2, MSH6, or PM22 loss</td>
<td></td>
</tr>
<tr>
<td>Data suggest chemoradiotherapy may influence MMR status via loss of MSH6 on IHC.</td>
<td>MSI-H</td>
<td></td>
</tr>
<tr>
<td>Sensitivity of MSI and IHC to detect LS in adenomas (~70%) may be reduced compared with CRC.</td>
<td>B-Raf V600E and methylation testing can separate sporadic from hereditary CRCs.</td>
<td>See Case 1</td>
</tr>
<tr>
<td>IHC may yield ambiguous results. Reasons include insufficient tissue and tumor fixation issues.</td>
<td>dMMR can be used to guide medical management in the absence of a detectable LS gene mutation.</td>
<td>See Case 2</td>
</tr>
<tr>
<td>If adenoma is the only tissue available for MMR testing, the additional sensitivity gained by pursuing molecular genetic testing must be weighed against its significant financial cost. This requires a detailed family history and thorough genetic counseling.</td>
<td>Screening should be pursued on pretreatment biopsy specimen if patient has undergone neoadjuvant chemoradiation. If not possible, interpret results with caution.</td>
<td>18, 57</td>
</tr>
<tr>
<td>If IHC results are equivocal, MSI testing is warranted. If result is MSI-H, further evaluation is necessary. Consider clinical genetics referral to assess the best approach.</td>
<td>dMMR in a non-CRC/EC could be used to guide genetic testing approach, but pMMR does not provide assurance that LS is NOT present. Consider clinical genetics referral.</td>
<td>58, 59</td>
</tr>
<tr>
<td>Abbreviations: AC-I, Amsterdam criteria I; CRC, colorectal cancer; dMMR, deficient mismatch repair; EC, endometrial cancer; IHC, immunohistochemistry; MMR, mismatch repair; MSI, microsatellite instability testing; MSI-H, high-level microsatellite instability; pMMR, proficient mismatch repair.</td>
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</table>

Figure 1 Reflex mismatch repair screening at Fox Chase Cancer Center.

Abbreviations: CRC, colorectal cancer; dMMR, deficient mismatch repair; EC, endometrial cancer; IHC, immunohistochemistry; MMR, mismatch repair; MSI, microsatellite instability testing; MSI-H, high-level microsatellite instability; pMMR, proficient mismatch repair.

*BRAF V600E analysis only in colorectal cancer, because this mutation seems to be absent in most endometrial cancers,

\[55,56\]
the same profile on MSI and immunohistochemical testing as the CRCs in individuals with LS caused by MLH1 gene mutations (MSI-H, loss of MLH1 and PMS2). This can complicate risk assessment.

In planning a testing strategy for this patient, the authors had 2 main testing options: assess for the presence of LS (MLH1 gene analysis), or test for evidence of a sporadic cause (tumor hypermethylation and/or BRAF V600E gene analysis). If the personal and family histories are consistent with LS, MLH1 analysis is a reasonable place to start, whereas if the patient has no family history or was not diagnosed at a young age, beginning with testing for hypermethylation and/or the BRAF V600E mutation may be less time-consuming and more cost-effective. Because of the number of family members affected with LS cancers, the authors chose to pursue MLH1 gene analysis first. Although no MLH1 mutation was identified, they could not assume LS was absent. To guard against the possibility of mutation undetected using standard testing techniques, they pursued evidence of an epigenetic cause for the dMMR results. Although the BRAF V600E mutation will not be found in all tumors with epigenetic silencing of MLH1, promoter methylation testing is technically complex and offered by few clinical laboratories. Therefore, the authors first pursued BRAF testing, which is available in their molecular laboratory, followed by methylation when the BRAF V600E mutation was not found. The patient’s positive hypermethylation results provide an answer for his loss of MLH1/PMS2 tumor results determined through MSI-H/immunohistochemical testing. The results also provide evidence that the underlying cause of his cancer was unlikely LS. Although coexistence of MLH1 hypermethylation and germline MLH1 gene mutations has been reported, this seems to be a rare occurrence and particularly unlikely in this patient given that MLH1 genetic testing showed no mutation.

**Clinical Implications:** Given his family history of CRC, the possibility of a different gene or combination of genes affecting risk in the family must be considered. The patient’s risk of a second primary CRC is difficult to quantify, but it would be reasonable for him and close relatives to be followed up closely with colonoscopy. The patient was advised to pursue a colonoscopy interval of 2 to 3 years, and his children were advised to begin colonoscopy in their 40s (10 years earlier than the patient), to be repeated every 3 to 5 years. He was advised to forego screening for the extracolonic cancers associated with LS, and was encouraged to inform the authors if he or family members developed other cancers or had more.

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Figure 2  Pedigrees for case 1 (A), case 2 (B), and case 3 (C). Abbreviations: CRC, colorectal cancer; DCIS, ductal carcinoma in situ.
than 10 cumulative adenomas on future screenings, because further testing (e.g., for an APC gene–associated polyposis) may then be justified.

Case 2

**Personal History:** A 61-year-old woman was referred for risk assessment because of a personal and family history of cancer. She was diagnosed with stage I endometrial cancer at 52 years of age (Figure 2B).

**Family History:** Paternal history included a father with transitional cell carcinoma of the renal pelvis (age, 69 years), an uncle with CRC (age, 65 years), an aunt with CRC (age, 57 years), and an aunt with gastric cancer (age, 60 years). Her paternal grandmother had endometrial cancer (age, 35 years).

**Testing Strategy:** The patient was counseled that her family history included a clustering of cancers typical for LS families, and further assessment for LS was recommended. MSI testing on the patient’s endometrial cancer showed a tumor that was MSI-H at 7 of 8 markers and showed loss of MSH2 and MSH6 on immunohistochemistry. This pattern of expression is consistent with LS, most frequently from a mutation in the MSH2 gene. Germline molecular analysis of MSH2 with reflex to MSH6 showed no mutation. When the patient was initially seen for risk assessment (in 2005), no further genetic testing was available. Because of her pattern of MSI and immunohistochemistry results in combination with her family history, she was given a putative diagnosis of LS and advised to follow associated screening guidelines. When clinical EPCAM testing became available, the patient was informed at her yearly clinic visit. She chose to pursue this testing, the results of which were negative (i.e., failed to detect a deletion).

**Discussion:** In contrast to Case 1, the underlying cause of dMMR is less uncertain in this patient. Even in the absence of a detectable LS mutation, the patient’s dMMR (MSI-H and loss of MSH2 and MSH6 on immunohistochemistry) indicates she has LS, but LS that is from a currently undetectable molecular cause. Unlike the dual loss of MLH1 and PMS2 on immunohistochemistry, loss of MSH2 and MSH6 expression does not commonly occur sporadically, nor does loss of MSH6 or PMS2 alone (see Table 2). Germline MSH2 promoter hypermethylation has been documented in the literature, but this does not seem to be common. This case shows the value of MMR screening with MSI or immunohistochemistry testing before germline DNA testing, and its ability to detect LS even when a gene mutation is not identified. If molecular genetic testing had been pursued first, the authors may have been tempted to consider the patient a phenocopy. Aside from family history, no evidence would have justified LS screening.

This case highlights recent updates in the understanding of LS. Since the patient’s initial evaluation in 2005, clinical LS genetic testing has expanded to include 2 additional genes (MSH6 and PMS2), and an entirely novel causative mechanism for the disease has been described (EPCAM).

**Clinical Implications:** Because the authors strongly suspect that this patient has hereditary dMMR because of clinical LS, appropriate medical management recommendations can be made for LS (for current recommendations, see the NCCN Guidelines for Colorectal Screening, available on the NCCN Web site at www.NCCN.org). She was advised that her first-degree relatives should consider themselves at risk for the currently undetected familial LS gene mutation.

Case 3

**Personal History:** A 49-year-old man was referred for risk assessment by his gastroenterologist because of a personal history of colon polyps and a family history of CRC. He had 3 polyps (nonadenomatous) removed when he was in his 40s (Figure 2C).

**Family History:** A sister died of CRC (diagnosed at 40 years of age), and another had 2 adenomas at 47 years of age. Paternal history included a father with CRC (age, 61 years), an aunt with CRC (age, 75 years), an uncle with CRC (age, 42 years), and a grandmother with CRC (age, 76 years).

**Testing Strategy:** The patient was counseled that his family represented a classic hereditary CRC kindred. The authors recommended he pursue MSI and immunohistochemical testing on his sister’s CRC to best define the family risk, but he expressed reluctance to involve his grieving brother-in-law, whose permission would be necessary to obtain the tumor specimen. The authors pursued germline genetic testing of MLH1, MSH2, and MSH6 (PMS2 testing was not yet available and EPCAM deletions had not been described) on the patient and this showed no mutation. The authors discussed with the patient that this was an uninformative result and reiterated the value of MSI and immunohistochemical testing on his sister’s tumor.

The authors drafted a letter for the patient to share with family explaining MSI and immunohistochemical testing and the potential implications. Af-
ter 1 year, his brother-in-law contacted the authors about pursuing MSI and immunohistochemical testing on his sister’s tumor. Immunohistochemical testing showed an MSS tumor with intact expression of MLH1, MSH2, MSH6, and PMS2.

Discussion: This case exemplifies an issue commonly encountered in cancer genetics: who is the best person to test in a family? Although testing the patient was convenient because he was unaffected, the authors’ only option was to pursue costly germline genetic testing for LS, which was likely to yield an uninformative result. His sister with adenomas was a slightly better candidate because, having manifested a clinical feature of LS, she was more likely to receive an informative result. Still, this would require involving an additional individual who may not be interested in testing or may face barriers to obtaining genetic services. Moreover, like the proband, even if no mutation was detected in her, this could not rule out LS, because she could be producing adenomas sporadically within an LS family. The ideal candidate for testing was his sister who was affected with CRC at 40 years of age. Identifying pMMR in an individual expressing the risk phenotype, (i.e., affected with CRC at a young age) is an excellent way to reduce suspicion of LS.

MSI and immunohistochemical testing on the tumor of a deceased individual is often overlooked as an option to define hereditary risk in a family. In this patient, the MSI and immunohistochemical test results were extremely valuable in elucidating cancer risks. Families meeting Amsterdam criteria I with MSS tumors have an overall lower CRC risk and later average age at diagnosis than individuals with LS; they also have no significantly elevated extracolonic cancer risks and do not demonstrate the rapid progression to CRC seen in MMR mutation carriers. This phenotype has been named familial colorectal cancer type X because the molecular origin has not yet been identified. The underlying genetic cause is likely heterogeneous, encompassing mutations in single genes with dominant inheritance, accumulation of risk from multiple low penetrance alleles, and shared environmental and familial influences in the family. Unfortunately, the authors cannot determine whether the original proband or his deceased sister’s daughters have inherited the predisposition to CRC, but more appropriate medical management recommendations can be made for the family.

Clinical Implications: A pMMR result on the proband’s sister’s tumor indicated that LS was an unlikely cause of the CRC phenotype in the family (although it remains possible that the sister was a phenocopy), and that extracolonic screening associated with LS was not warranted. Young individuals were advised to begin colonoscopy at 30 years of age (10 years before the earliest family diagnosis), and that screening frequency should reflect the hereditary risk in the family (the recommendation was for colonoscopy every 2 to 3 years, although a very conservative 1-year interval could also be considered despite an unknown molecular risk). Depending on the findings of future colonoscopies, the proband or other family members may be candidates for additional genetic testing.

Conclusions
Identifying LS remains a public health imperative. Recommendations and clinical strategies for more-expansive universal LS screening and an ever-expanding repertoire of molecular testing options are emerging and have the potential to improve LS detection. General and specialty providers need a better understanding of this common cancer risk syndrome; how to best identify clinically affected individuals, mutation carriers, and family members; and the medical implications, including recommended preventive screening and surgical prophylaxis.

References
Genetic Testing for Lynch Syndrome


