

## Diagnosing Lynch Syndrome: More Light at the End of the Tunnel

Randall W. Burt

### Abstract

Since the recognition of Lynch syndrome, which confers a high risk of colorectal, uterine, and other cancers, approaches to its diagnosis have included a family history of associated cancers and web-based algorithms. Identification of causative genes now allows a precise diagnosis, thus focusing present efforts on who should have genetic testing. Testing for cancer tissue changes can determine who should have germline genetic testing. Indeed, such tumor testing is now generally recommended for all newly diagnosed colorectal cancer cases. As reported in this issue of the journal by Yurgelun and colleagues (beginning on page 574), large colorectal adenomatous polyps ( $\geq 10$  mm) from patients with Lynch syndrome exhibit findings similar to those in Lynch syndrome colorectal cancer tissues. This finding indicates that testing larger adenomas in persons at a significant risk for Lynch syndrome can now determine the need for germline genetic testing. Although further study is needed for general application, the present study justifies large polyp testing in high-risk families when cancer tissue is unavailable, albeit negative polyp tissue would not rule out Lynch syndrome, as would negative cancer tissue. *Cancer Prev Res*; 5(4); 507–10. ©2012 AACR.

Lynch syndrome is one of the most commonly inherited cancer conditions, accounting for 2% to 4% of colorectal cancer (CRC) cases. The population frequency of Lynch syndrome is slightly more than one in 500 individuals (1). In addition to a 50% to 80% lifetime risk of CRC, patients with Lynch syndrome have a 40% to 60% risk of uterine cancer and an elevated risk of ovarian, pancreatic, gastric, upper-urinary tract, renal, biliary, small bowel, and central nervous system (CNS) malignancies. Colorectal surveillance of persons with this condition leads to greatly reduced CRC incidence and mortality. Appropriately timed hysterectomy and ovariectomy likewise results in a reduced incidence of malignancies in these organs. The diagnosis of Lynch syndrome is thus critical to the prevention and early detection of cancer in affected persons and families, notwithstanding that the effectiveness of screening patients with Lynch syndrome for noncolorectal and nonuterine malignancies remains uncertain. As will be discussed later, the article by Yurgelun and colleagues in this issue of the journal provides an important new tool for the diagnosis of Lynch syndrome (2).

The major issue in Lynch syndrome remains failure to diagnose for a variety of reasons. The lack of a distinctive

phenotype in affected persons is perhaps the greatest reason for this failure. Other autosomal dominantly inherited CRC syndromes, such as familial adenomatous polyposis, Peutz-Jeghers syndrome, and juvenile polyposis, are characterized by numerous and often histologically distinctive gastrointestinal polyps, as well as by distinctive extraintestinal findings (1). Although Lynch syndrome colorectal polyps and cancers occur on average at younger ages, the age range substantially overlaps that of sporadic counterparts. The "polyp to cancer" sequence also appears to be greatly accelerated in Lynch syndrome neoplasia (3), but this is not distinguishable for diagnosis. Last, it appears that family history is often neglected in medical evaluations.

A number of approaches have been developed to address the difficulty of diagnosing Lynch syndrome (1). The first established diagnostic approach was the Amsterdam criteria, which are as follows: (i) a patient with CRC must have 2 first-degree relatives with CRC; (ii) at least 2 successive generations must be affected with CRC; and (iii) one person must be diagnosed with CRC under the age of 50 years. These criteria are quite specific but not very sensitive, missing at least half of the people and families with Lynch syndrome. In an attempt to improve the sensitivity, Amsterdam II criteria were developed. These are the same as the original Amsterdam criteria but for replacing CRC with any Lynch syndrome-related malignancy. The Amsterdam II criteria have proved to be more sensitive but are correspondingly less specific.

Several algorithms that involve a family history of Lynch-associated cancers have been developed to determine the likelihood of Lynch syndrome. Each of these algorithms,

**Author's Affiliation:** Department of Medicine, School of Medicine, Huntsman Cancer Institute, University of Utah, Salt Lake City, Utah

**Corresponding Author:** Randall W. Burt, Department of Medicine, Huntsman Cancer Institute, University of Utah, 2000 Circle of Hope, Salt Lake City, UT 84112. Phone: 801-585-3281; Fax: 1-801-581-3389; E-mail: randall.burt@hci.utah.edu

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including MMRpredict (MMR, mismatch repair), Leiden, MMRPro, and PREMM<sub>1,2,6</sub> (4), is available online, takes only minutes to complete, and does well in assessing the risk of Lynch syndrome (5). The group that developed PREMM<sub>1,2,6</sub> (6) also examined which questions are most reliable in predicting Lynch syndrome (7). The 3 most informative questions they found are as follows: "Do you have a first-degree relative with CRC or a Lynch syndrome-related cancer diagnosed before the age of 50?"; "Have you had CRC or polyps diagnosed before the age of 50?"; and "Do you have 3 or more relatives with CRC?" These questions identified 77% of all high-risk individuals and 95% of Lynch syndrome mutation carriers.

Another approach for diagnosing the syndrome came from the identification of its molecular genetic pathogenesis (4). Lynch syndrome arises from disease-causing mutations in one of the 4 MMR genes mutL homolog 1 (*MLH1*), mutS homolog 2 (*MSH2*), *MSH6*, and *PMS2*. The first 2 genes account for more than 90% of cases, *MSH6* for about 6%, and *PMS2* for 1% to 2%. Various types of mutations of the epithelial cell adhesion molecule (*EpcAM*) gene, which is directly adjacent to *MSH2*, can affect *MSH2* translation and thus cause MMR dysfunction (1, 4, 8). MMR genes repair certain types of DNA copying errors, and their dysfunction frequently causes DNA loss in short-segment DNA repeats, called microsatellites. An established panel of 5 of these microsatellites indicates microsatellite instability (MSI) when 2 or more of the microsatellites are mutated. MSI is a feature of nearly all colorectal and uterine cancers that occur in Lynch syndrome. Therefore, tumors that exhibit MSI must be suspected of arising from Lynch syndrome, and the patient should undergo germline genetic testing to identify the disease-causing mutation.

Unfortunately, MSI is also found in about 10% of sporadic CRCs. In the sporadic setting, MSI is almost always caused by somatic methylation of the *MLH1* promoter, leading to gene dysfunction. Thus, only about 15% to 20% of MSI-positive CRCs arise from Lynch syndrome (9). One can narrow the group needing germline genetic testing by first testing the tumor for methylation of the *MLH1* promoter or for a specific mutation in *BRAF* that indicates hypermethylation. Either tumor test will produce positive results that will eliminate about half of the candidates for germline genetic testing. MSI tumor testing has proven to be 90% to 95% sensitive for finding Lynch syndrome. It should also be noted, however, that although MSI-negative CRCs almost never arise from Lynch syndrome, there are exceptions. Mutations in *MSH6* or *PMS2* can give rise to a few MSI-negative Lynch syndrome CRCs (along with MSI-positive Lynch syndrome CRCs). Interestingly, a specific mutation in *MLH1* also has been found to give rise to MSI-negative tumors by eliminating *MLH1*-FANCD1 binding, which in turn delays MMR signaling, allowing reverse DNA methylation (10).

The next issue is, which patients should have their CRCs tested for MSI? The Bethesda guidelines were established to identify these patients (1). Under the Bethesda guidelines, colorectal tumor testing for MSI should be done if any one

of the following criteria are met: CRC diagnosed at less than 50 years old; the presence of synchronous or metachronous CRC or of other Lynch syndrome-associated cancers, regardless of age; in a patient less than 60 years old, CRC diagnosed with "MSI histology" (1); CRC diagnosed in an individual and 1 or more first-degree relative with a Lynch syndrome-associated tumor, with at least one of the cancers being diagnosed at less than 50 years old; and CRC diagnosed in an individual and 2 or more first- or second-degree relatives with Lynch syndrome-associated tumors, regardless of age. Although these guidelines are quite sensitive for finding Lynch syndrome cases, their complexity has resulted in infrequent use in the clinical setting.

Another tumor test similar in sensitivity and specificity to MSI tumor testing is immunohistochemical testing for expression of MMR proteins, hereafter abbreviated to "immunohistochemical testing" (4). Lack of expression of any of the MMR proteins indicates underlying mutation or dysfunction of the related MMR gene. An advantage of immunohistochemical testing is that it can indicate which specific MMR gene should be examined for germline mutation. But if *MLH1* is underexpressed, then methylation testing should be done, similar to that outlined in connection with MSI tumor testing, before germline genetic testing is done. One problem with immunohistochemical testing, however, is that the immunohistochemistry for MMR protein expression is somewhat difficult, requiring considerable experience in carrying out the test for optimal results.

Once tumor testing indicates that germline genetic testing should be done, DNA is usually obtained from peripheral white blood cells. Most genetic testing laboratories sequence the 4 MMR genes, beginning with *MLH1* and *MSH2*. If immunohistochemical tumor testing indicates a specific gene, then only that gene is examined. If no mutation is found by sequencing, further tests to identify large deletions or rearrangements are done. A major benefit to individuals and families is that mutation-specific testing can be done in any family member with nearly 100% accuracy after the disease-causing mutation is found in the index case. Furthermore, mutation-specific testing is a fraction of the cost of the initial testing for mutation.

If MSI or immunohistochemical tumor testing is done to select candidates for germline genetic testing, disease-causing genetic mutations will be found in the large majority of selected cases. Genetic testing based on Amsterdam criteria alone results in the finding of mutations in about half of the cases. Families that meet Amsterdam criteria but do not have a mutation appear usually not to have Lynch syndrome. They have an increased risk of CRC, but usually not the other malignancies found in Lynch syndrome. The genetic cause or causes of this category of families remains to be elucidated. These families should probably undergo tumor testing to confirm lack of MSI or immunohistochemical findings and should be followed and surveilled as high CRC risk families without Lynch syndrome.

Immunohistochemical and/or MSI testing in all newly diagnosed CRCs is a recent approach for determining who

should have genetic testing (4). This approach eliminates the complexity of following the Bethesda guidelines and has been shown to be more complete in detecting Lynch syndrome cases and families than are Amsterdam I or II criteria or even the Bethesda guidelines. It also eliminates the exercise of using the online risk tools to determine who should be tested. A recent modeling study examined all of the major approaches outlined earlier for diagnosing Lynch syndrome—Amsterdam criteria, Bethesda guidelines, online modeling algorithms, and testing all patients with CRC—to see which is the most medically effective and most cost-effective (11). The study successively modeled each approach on the same hypothetical CRC case. It also examined with each of the approaches whether proceeding directly to germline genetic testing (after suspicion of Lynch syndrome) or whether conducting immunohistochemical tumor testing to indicate who should have germline testing was more effective.

The modeling found that the most effective approach was tumor immunohistochemical testing for all patients with CRC, followed by *BRAF* testing when immunohistochemical showed an abnormally low expression of MLH1, followed by germline genetic testing when indicated by the previous tests. Other studies and health policy organizations also support this approach, leading to a consensus that tumor testing is an optimal first approach that should be recommended for all cases of newly diagnosed CRC (4, 9, 12–16). The cost-effectiveness of any approach was very sensitive to how many relatives were tested by mutation-specific testing after a disease-causing mutation was found in the index case. It seems that MSI or immunohistochemical testing in uterine cancers may be as effective as in CRCs and therefore should also be considered in evaluating persons and families for Lynch syndrome (17, 18). Whether tumor testing in other (nonuterine or noncolorectal) Lynch-associated malignancies would be as effective is unclear.

A remaining issue is how to determine when genetic testing for Lynch syndrome should be done in people with a strong family history of syndrome-related cancers but no cancer themselves. A recent modeling study by Dinh and colleagues examined direct-to-germline genetic testing for such individuals based on the risk calculated for Lynch syndrome by the PREMM<sub>1,2,6</sub> model (19). They found that genetic testing starting at age 25 to 35 years in patients with a 5% or greater risk of Lynch syndrome was both medically effective and cost-effective for diagnosis, even compared with the now standard practice of tumor screening by immunohistochemical and/or MSI first.

As indicated earlier, there is a growing consensus that the preferred approach to diagnosing Lynch syndrome is to conduct immunohistochemical and/or MSI testing on all newly diagnosed CRCs and probably uterine cancers, followed by germline genetic testing as indicated. If a substantial risk of Lynch syndrome is present in the family based on family history criteria, but the patient in question does not have cancer, then tumor tissue from a relative should be

tested. If this tissue is not available, then direct-to-genetic testing seems to be appropriate for patients with a 5% or more risk for Lynch syndrome, as determined by family history (e.g., through applying the Amsterdam criteria) or as calculated by an online risk model.

The study in this issue of the journal by Yurgelun and colleagues adds considerably to the currently preferred approach by showing that larger colorectal adenomatous polyps from patients with Lynch syndrome usually exhibit MSI or immunohistochemical abnormalities (2). They found MSI and abnormal immunohistochemical in most polyps 8 mm or more and in all polyps 10 mm or more in diameter. Smaller adenomas did not reliably show abnormalities. Many patients with a strong family history of CRC, but no cancer history themselves, undergo surveillance colonoscopy that finds adenomatous polyps. A frequent surveillance-related question is whether testing the polyps for MSI or immunohistochemical abnormalities, especially when cancer tissue is not easily available, might help to determine the need for germline genetic testing. This new study indicates that MSI and/or immunohistochemical testing in adenomatous polyps 10 mm or more indeed would be helpful in this setting. The study does not determine, however, if failure to find MSI or immunohistochemical abnormalities in larger adenomas rules out the diagnosis of Lynch syndrome, as it would in cancer. It also does not indicate whether testing for MSI and/or immunohistochemical should be conducted in all larger adenomatous polyps found in general screening populations. Larger prospective studies of consecutive polyps are needed to answer these questions.

For now, adenomatous polyp testing for MSI and immunohistochemical abnormalities to consider germline testing for Lynch syndrome should be limited to families with a strong family history of Lynch syndrome-associated cancers and limited to adenomas 10 mm or more in diameter. Additional studies are also needed to examine the cost and medical effectiveness of testing larger adenomatous polyps in populations with less risk. But for the present, if CRC tissue is not available from the patient (or a relative) in a family where the presence of Lynch syndrome is being considered, testing an adenomatous polyp 10 mm or more seems justified.

Approaches to genetic testing for inherited cancer syndromes are evolving rapidly. A number of laboratories are already developing panels of inherited cancer genes, for which testing likely will be less expensive than is present cancer syndrome-specific testing. The availability of such panel tests may well change the algorithms for assessing the medical effectiveness and cost-effectiveness of various approaches for diagnosing cancer syndromes. Next generation sequencing also may soon be available for individual testing at reasonable costs. Such new testing developments may well make direct-to-genetic testing based on risk increasingly attractive. At some point, general testing for an inherited syndrome without regard to risk may even become a reality, which

would get the diagnosis of Lynch syndrome out of the tunnel altogether.

#### Disclosure of Potential Conflicts of Interest

R. Burt is a consultant/advisory board member for Myriad Genetics, Inc.

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#### Authors' Contributions

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Randall W. Burt

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