The Genetics of Hereditary Non-Polyposis Colorectal Cancer

Stephen B. Gruber, MD, PhD,*† and Wendy Kohlmann, MD,* Ann Arbor, Michigan

**Keywords**
Hereditary nonpolyposis colorectal cancer, genetic testing, Lynch syndrome

**Abstract**
Hereditary nonpolyposis colorectal cancer (HNPCC) is a common autosomal dominant cancer syndrome characterized by an inherited predisposition to a wide spectrum of cancers, typically presenting at a relatively early age. HNPCC is also commonly described as Lynch syndrome, recognizing the invaluable contributions of Dr. Henry Lynch to the clinical characterization of this entity. Historically, the term Lynch syndrome type I referred to HNPCC with a phenotypic spectrum limited to colorectal cancer, and Lynch syndrome type II described HNPCC in association with extracolonic malignancies. Because researchers have shown the molecular basis of both variants of HNPCC to be related to mutations in mismatch repair genes, these distinctions are generally no longer emphasized.

Of historical interest, Lynch’s initial descriptions extended the findings from a report in 1913 by Dr. Aldred Warthin at the University of Michigan. This large family showed clear evidence of autosomal dominant transmission of a spectrum of cancers, dominated by early onset cancers of the stomach, colon, and uterus. Through subsequent generations within this family, cancers of the stomach have become less common, corresponding with the decline in stomach cancer incidence in the United States over the past 100 years. This first family of HNPCC highlights the 3 hallmarks of HNPCC: a striking family history of cancer consistent with autosomal dominant inheritance, young ages at cancer diagnosis, and the presence of a recognizable spectrum of multiple primary cancers. This family continues to be followed up by Dr. Lynch and our group at the University of Michigan.

Diagnostic criteria for HNPCC were first established in 1990 by the International Collaborative Group on Hereditary NonPolyposis Colorectal Cancer to optimize family selection for positional cloning of the responsible genes. These criteria, called the Amsterdam criteria (Table 1) take advantage of the features noted by Lynch, and can commonly be remembered as the “3-2-1-0” rule. Families meet classic Amsterdam criteria for HNPCC when 3 or more families members have histologically verified colorectal cancer (related to one another through a first-degree relative); 2 or more generations are involved, with at least 1 individual diagnosed before age 50; and 0 individuals have FAP.

Although highly specific, the classic Amsterdam criteria were revised to improve clinical sensitivity by incorporating other elements of the cancer phenotype in the
The modified Amsterdam criteria (Table 2) rely on the histologic diagnosis of HNPCC-associated cancers, expanded to include cancer of the endometrium, ureter, renal pelvis, and small bowel. Interestingly, this expanded definition does not include the full spectrum of cancers seen in HNPCC, but merely highlights the tumors most informative for facilitating a clinical diagnosis. With this revision, the diagnosis of colorectal cancer is included, but not required to fulfill the modified Amsterdam criteria (Amsterdam II).

In addition, a National Cancer Institute (NCI) consensus conference established guidelines to improve the clinical recognition of HNPCC through microsatellite instability testing. The Bethesda guidelines (Table 3) include criteria based on age, pathologic characteristics, family history, and the presence of extracolonic cancers to identify patients who should be considered for a molecular diagnostic workup for HNPCC. Elements of the Bethesda guidelines take advantage of the well-known predisposition to right-sided colon cancers, as well as cancer with specific histopathologic features such as poor differentiation and a solid or cribriform histologic pattern.

Other important histologic clues suggestive of HNPCC, such as the presence of tumor-infiltrating lymphocytes, are not incorporated into the Bethesda guidelines. A medical position statement by the American Gastroenterological Association recommended a modified version of the Bethesda guidelines that changes the criteria for age of cancer diagnosis to less than 50, which should improve the clinical sensitivity for recognizing HNPCC even further.

The full spectrum of tumors seen in HNPCC includes colorectal adenocarcinoma, endometrial adenocarcinoma, transitional cell carcinoma of the bladder, urethra, and renal pelvis, and adenocarcinomas of the stomach, small bowel, ovary, and biliary tract. Brain cancer is a rare complication of HNPCC, and this recognized association between glioblastoma and HNPCC is called Turcot syndrome. Sebaceous gland adenomas and carcinomas and keratoacanthomas are seen in the Muir-Torre variant of HNPCC.

<table>
<thead>
<tr>
<th>Table 1 Original Amsterdam Criteria (I) for HNPCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Three or more relatives with histopathologically verified colorectal cancer, one of whom is a first-degree relative of the other two</td>
</tr>
<tr>
<td>• At least two successive generations should be affected</td>
</tr>
<tr>
<td>• At least one colorectal cancer should be diagnosed before age 50</td>
</tr>
<tr>
<td>• FAP should be excluded (0 individuals with FAP)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2 Revised Amsterdam Criteria (II) for HNPCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Three or more relatives with histopathologically verified HNPCC-associated cancer (colorectal cancer, cancer of the endometrium, small bowel, ureter, or renal pelvis), one of whom is a first-degree relative of the other two</td>
</tr>
<tr>
<td>• At least two successive generations should be affected</td>
</tr>
<tr>
<td>• At least one colorectal cancer should be diagnosed before age 50</td>
</tr>
<tr>
<td>• FAP should be excluded (0 individuals with FAP)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 3 Bethesda Guidelines for Evaluating Colorectal Tumors For Microsatellite Instability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Individuals with cancer in families that meet the Amsterdam criteria</td>
</tr>
<tr>
<td>2. Individuals with 2 HNPCC-related cancers, including synchronous and metachronous colorectal cancers or associated extracolonic cancers</td>
</tr>
<tr>
<td>3. Individuals with colorectal cancer and a first-degree relative with colorectal cancer or HNPCC-related extracolonic cancer or a colorectal adenoma; one of the cancers diagnosed &lt; age 45, and the adenoma diagnosed &lt; age 40</td>
</tr>
<tr>
<td>4. Individuals with CRC or endometrial cancer diagnosed at age &lt; 45 (now revised as age 50)</td>
</tr>
<tr>
<td>5. Individuals with right-sided colorectal cancer with an undifferentiated pattern (solid/cribriform) on histopathology diagnosed &lt; age 45</td>
</tr>
<tr>
<td>6. Individuals with signet-ring-cell type CRC diagnosed &lt; age 45</td>
</tr>
<tr>
<td>7. Individuals with adenomas diagnosed &lt; age 40</td>
</tr>
</tbody>
</table>

* Endometrial, ovarian, gastric, hepatobiliary, or small-bowel cancer or transitional cell carcinoma of the renal pelvis or ureter.
* Solid/cribriform defined as poorly differentiated or undifferentiated carcinoma composed of irregular, solid sheets of large eosinophilic cells and containing small gland-like spaces.
* Composed of >50% signet ring cells.
Cumulative lifetime risks of cancer have been estimated in several studies, although few of these have been population based. An important limitation of penetrance estimates from registries is ascertainment bias; therefore, the registry-based figures cited here probably represent the upper limits of penetrance. The lifetime risk of colorectal cancer in HNPCC is approximately 80%,\(^1\,^3\) and the lifetime risk of endometrial cancer ranges from 20% to 60%, depending on the registry.\(^1\,^4,^5\) The Finnish registry has completed some of the most detailed analyses by estimating the cumulative lifetime risk of several cancers to age 70, with stomach cancer between 13% and 15%, ovarian cancer between 9% and 12%, biliary tract cancers between 2% and 7%, urinary tract cancers between 4% and 5%, and brain cancers between 1% and 3.7%\(^1\,^5,^6\).

### Molecular Genetics of Mismatch Repair

Cancer is a disease of DNA, and mismatch repair is critical to maintaining the fidelity of DNA to protect cells from malignant transformation. During DNA replication, DNA polymerases commonly introduce single nucleotide mismatches as well as small insertion or deletion loops (IDLs); however, with normal mismatch repair these errors are corrected and interrupted before they can clonally proliferate through subsequent cell divisions.\(^1\,^7\) When the mismatch repair system is defective, these types of somatic errors are permitted to accumulate within tumor-suppressor genes and oncogenes at an accelerated pace.

A molecular hallmark of HNPCC is microsatellite instability,\(^6,^1\,^8,^1\,^9\) which arises on the basis of unrepaired mismatches and IDLs. Microsatellite instability testing is performed by extracting DNA from microdissected tumor and adjacent normal epithelium (Fig. 1). A standard panel of anonymous DNA markers has been recommended to evaluate microsatellite instability in colorectal cancer (Table 4).\(^6\)

Taking advantage of fundamental investigations of mismatch repair in yeast, researchers localized and cloned the human mismatch repair genes by recognizing the relationship between microsatellite instability and HNPCC.\(^1\,^8,^1\,^9,^1\,^2\) MSH2 is the human homolog of the yeast gene \textit{mutS} and was simultaneously identified by several groups in 1993 as a gene responsible for HNPCC.\(^2\,^1,^2\,^2\) Within a year, a second human mismatch repair gene, MLH1, was cloned.\(^2\,^1,^2\,^4\) Subsequent work has identified other genes within the mismatch repair system, including MSH6.

The molecular mechanisms of defective mismatch repair are now also becoming clear. In an elegant paper by Lamers et al.,\(^1\,^7\) the crystal structure of the MutS dimer protein was captured while binding to a G*T mismatch. To quote from their report, “The MutS dimer is an asymmetric molecule that looks like a pair of praying hands, with the wrists close together, the thumbs coming close and the fingers lightly touching. The DNA is held between the thumbs and fingers in this view.”\(^1\,^7\) A sliding clamp model was proposed in humans that may be briefly described as the formation of a heterodimer between two of the human mutS homologs (MSH2 and MSH6, for example), that recognize DNA

---

**Figure 1** Microsatellite instability testing. Recut sections of tumor blocks are evaluated by a pathologist to designate areas of normal epithelium and cancer for microdissection. These areas are microdissected, typically by carefully scraping with a razor. DNA is extracted separately from each microdissected region, is polymerase chain reaction-amplified using a standard panel of markers (Table 4), and run on a gel. With normal replication and intact mismatch repair, fidelity of the genomic DNA sequence is maintained. The sequence can be recognized by similar electrophoretic patterns showing maternal and paternal alleles. With defective mismatch repair, shown on the right, insertion deletion loops are inconsistently repaired. This is manifested by extra bands seen on gel electrophoresis. The arrow on the right side of the gel highlighting the extra lower band represents a shorter fragment of DNA introduced by the failure to repair a short deletion.
mismatches in DNA sliding between the heterodimer. Mismatches provoke the binding of mutL homologs to facilitate DNA repair.25

The critical clinical feature of the defective mismatch repair is that this special class of tumor-suppressor genes normally functions to maintain genetic fidelity and generally protects cells from cumulative DNA damage over time. When both copies of a mismatch repair gene are inactivated, typically through germline mutation of one copy and somatic inactivation of the other, DNA mutations accumulate rapidly in many other genes that are critical to the regulation of cellular growth and death. Thus it is not uncommon for the entire sequence of accumulated genetic mutations required to transform normal epithelium through its intermediate precursors all the way to cancer within 1 to 3 years with defective mismatch repair. This is in striking contrast to the development of sporadic colorectal cancer, which is thought to typically take 10 or more years.

**Genetic Testing**

Although HNPCC may be caused by mutations in any 1 of 5 different mismatch repair genes, MLH1, MSH2, PMS1, PMS2, or MSH6,26 mutations in MLH1 and MSH2 account for the majority of cases.27 Mutations in MSH6 may account for up to 10% of HNPCC families, but the evidence for a role of PMS1 and PMS2 in HNPCC is largely anecdotal. Some MSH6 mutations have been identified in families who do not exhibit classic HNPCC features, suggesting that MSH6 mutation families may be characterized by microsatellite low instability (MSI low) or microsatellite stable (MSS) tumors, lower cancer penetrance, and later age of onset.26,27 Mutations in PMS1 and PMS2 have been reported to be associated with a few HNPCC families. However, other studies have failed to confirm a significant role for these genes in HNPCC.30

Reports of the mutation detection rates of genetic testing vary widely depending on the criteria used for the selection of the study population and the testing methodology. Evaluations of the sensitivity and specificity of various criteria have found that the Amsterdam criteria have the highest specificity. However, these restrictive criteria can miss 39% of families with mutations. A sensitivity of 94% has been reported for the Bethesda criteria; however, a specificity of only 25% was also reported.31 Verification of reported family history is necessary to accurately assess the risk for HNPCC. Kateballe et al32 reported a 21% false-positive rate for the Amsterdam criteria and a 39% false-negative rate based on inaccuracies in the patient’s verbal report of family history.

The use of MSI and immunohistochemistry may help more accurately target individuals who are likely to carry germline mutations. A study conducted by Cunningham et al33 reported a high rate of mutation detection in individuals whose tumors were MSI-H and exhibited loss of MSH2 staining. However, the incidence of germline mutations among those whose tumors demonstrated a loss of MLH1 protein expression was low. The majority of these cases were due to hypermethylation of the MLH1 promoter rather than a germline mutation.

Clinical genetic testing is commercially available for MLH1 and MSH2 through several different laboratories. Some labs may use single-strand conformational polymorphism or denaturing gradient gel electrophoresis. These tests detect changes in the pattern of DNA migration through a gel, as an initial mutation screen. The region of the gene identified through this initial step as the area likely to harbor the mutation is then sequenced to identify the specific mutation. Full sequencing is considered to be the gold standard for mutation testing,33 although this technique will miss large deletions. Sequencing involves analyzing each base pair in the coding region and those delineating exon boundaries. Currently the cost of genetic testing of the
MLH1 and MSH2 genes varies from $1,300 to $1,950. Once a mutation has been identified, at-risk family members can be tested for the specific mutation for approximately $300.14 At the time of this article, one commercial laboratory that was providing clinical genetic testing for MSH6 was identified through http://www.genetests.org.

Current approaches to testing will miss large deletions and other genomic rearrangements.34 Up to 20% of families meeting Amsterdam criteria may have genomic rearrangements in MSH2.36 Conversion technology allows for alleles to be separated and analyzed individually. This technology has been shown to improve mutation detection rates.17 At least one clinical laboratory is currently offering Southern blot analysis of MLH1 and MSH2 for patients who do not have an alteration identified through the initial confirmation gel electrophoresis screening, to help identify patients with large deletions.

Genetic counseling is recommended as an initial step for people considering the option of genetic testing.38 The process of genetic counseling includes providing risk assessment, educating patients about testing options, discussing the implications for medical management, and facilitating the psychosocial adjustment of the individual and family.39,40

The development of predictive testing for hereditary cancer syndromes raised concerns about the potential effects of knowing risk status on mental health. However, studies that have evaluated levels of distress among individuals undergoing predictive genetic testing for other hereditary cancer syndromes have not found genetic testing to be associated with adverse psychological effects.41-43 However, individuals with high levels of distress before testing, those who lack social support and quality of life, and those who question their ability to cope with a positive test result are at higher risk for psychological problems.44-46 Identifying individuals who may be at high risk for distress before testing will allow them to be directed to additional support services.

Prognosis
Colon cancers related to HNPCC are associated with a better prognosis and overall survival than sporadic colon cancers matched for stage.47 This is a paradoxical finding, because HNPCC-related colon cancers are often poorly differentiated, which is usually an indicator of poor prognosis. The presence of MSI in the tumor tissue has also been shown to be a predictor of better prognosis among patients with sporadic colon and endometrial cancers.48-50 Watanabe et al.49 conducted a study involving 656 colorectal cancer patients and found that adjuvant chemotherapy was associated with improved survival in patients with microsatellite unstable tumors over patients with microsatellite stable tumors.

Screening
As noted previously, HNPCC is associated with an 80% lifetime risk of colorectal cancer with an average age of diagnosis at 44.40 Regular colonoscopy with removal of precancerous polyps has been found to reduce the incidence of colon cancer in individuals with HNPCC.51 Experts recommend that individuals at risk for HNPCC undergo colonoscopy every 1 to 2 years beginning between 20 and 25 years of age, or 10 years younger than the earliest age at diagnosis in the family, whichever is earlier. Colonoscopy should be repeated annually beginning at age 40. Full colonoscopy rather than a flexible sigmoidoscopy is recommended, because approximately 70% of colon tumors occur in the proximal colon.13

Prophylactic colectomy is not generally recommended for HNPCC mutation carriers; however, if a cancer is detected, a full colectomy with ileorectal anastomosis is recommended rather than a resection due the high risk of metachronous cancers.16,52 Many patients are not evaluated for HNPCC until after the treatment of their initial cancer, and many of these high risk patients may have been treated only with a resection. Referring patients diagnosed with colon cancer at a young age or those who have a strong family history for a genetics evaluation before surgery can help determine the optimal surgical approach.

Screening approaches for endometrial and ovarian cancer are less well established than those for colon cancer. In addition to an annual Pap smear and pelvic examinations, at-risk women may also consider an annual transvaginal ultrasound, endometrial biopsy, and CA-125 blood test to screen for endometrial and ovarian cancer.53 Dove-Edwin et al.54 recently reported on the use of transvaginal ultrasound to screen for endometrial cancer in women at risk for HNPCC. No cancers were detected in the screening; however, 2 incident cancers did develop during the course of the study. The diagnoses were made at an early stage based on symptoms.

No data from studies evaluating the combination of transvaginal ultrasound and endometrial biopsy
have been reported. Prophylactic total abdominal hysterectomy with salpingo-oophorectomy is not routinely recommended as a preventive measure but may be considered by women who have completed child bearing. General population studies have shown that the use of combined oral contraceptives is associated with reduced endometrial and ovarian cancer risks. Currently studies have been funded to begin investigating where the same protective effect will be seen in women with HNPCC.

Additional screening recommendations for other HNPCC-related cancers have not been established. Periodic upper endoscopy surveillance is an option to screen for gastric and duodenal cancers. However, a recent study suggested no benefit from upper endoscopy screening in mutation carriers because of a lack of precursor lesions. Annual urine cytology is an approach for screening for HNPCC-related urinary tract cancers. There is not clear evidence available regarding the effectiveness of urine cytology in individuals with HNPCC.

Although screening has been shown to be an effective tool for reducing colon cancer incidence in families with HNPCC, adherence to recommendations is lacking. A study of individuals undergoing genetic counseling and testing found that 45% to 48% had never undergone colonoscopy, and 28% had never undergone any type of colon cancer screening. The process of genetic counseling and testing may help increase adherence, with 70% of patients reporting the intention to screen following disclosure of test results. However, long-term studies are needed to determine factors affecting long-term adherence to screening guidelines.

Conclusions

HNPCC represents a common cancer syndrome that can be readily recognized by clinical, pathologic, and molecular features. Genetic testing is rapidly becoming the standard of care to guide the treatment of patients and their families, and molecular diagnostic workups have been shown to be cost effective. Even more importantly, screening for colorectal cancer with colonoscopy beginning between the ages of 20 and 25 has been shown to substantially reduce the incidence and mortality from colorectal cancer. Data suggest that some of the extracolonic cancers associated with HNPCC are likely to benefit from enhanced surveillance, chemoprevention, and risk-reducing surgery, and future studies need to examine approaches to the integrated management of individuals at risk of HNPCC.

References

3. Warthin AS. Heredity with reference to carcinoma as shown by the study of the cases examined in the pathological laboratory of the University of Michigan, 1895–1913. Arch Intern Med 1913;12:546–555.
Genetics of HNPCC


