Inherited Colon and Rectal Cancer: Surgical Perspectives

Part 1—Hereditary Nonpolyposis Colorectal Cancer: Genetics and Diagnosis

THE ALPHABET SOUP
of Genetics and Diagnosis Explained

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With a myriad of vexing abbreviations and obscure terminology, the genetics controlling the formation of colonic polyps and malignancies may be difficult to appreciate. Paradoxically, the physician, positioned at the beginning of the diagnostic effort, is often the person most hampered by a lack of basic understanding of this genetic alphabet soup. All that is required to overcome this obstacle is a refresher in basic genetics and a high index of clinical suspicion. As the science underlying the development of inherited colorectal cancer has become better understood, the clinician has become better equipped to stand at the forefront of the diagnostic and treatment effort.

In Part 1 of this series, we will examine the roles of the pathologist and the geneticist in diagnosing hereditary nonpolyposis colorectal cancer. In Part 2, we will discuss screening and treatment and the roles of the epidemiologist, the diagnosticians and the surgeon. In Parts 3 and 4, we will address familial adenomatous polyposis.

Sporadic, Familial and Inherited Disease

Of all colon cancers, 80% to 90% occur *sporadically,* with no known etiology. Ten percent to 15% of patients have *familial* colorectal cancer, meaning that there are two or more colorectal malignancies found in a given family and that a specific causative gene has not been identified. *Inherited* or *hereditary* forms of colon cancer account for 5% of cases, in which a causative genetic abnormality has been found to be associated with the malignancy.

Generally, inherited colorectal cancers are divided into two groups. The first group is composed of malignancies arising in a background of epithelial polyposis or hamartomatous colonic polyps. Familial adenomatous polyposis is the most common syndrome in this group. The second group is represented by malignancies arising in grossly normal-appearing mucosa with few or no visible underlying polyps. Hereditary nonpolyposis colorectal cancer (HNPCC) is the most common syndrome in this group.

Hereditary Nonpolyposis Colorectal Cancer

HNPCC, or Lynch syndrome, accounts for 4% of all colorectal cancers. Originally characterized in 1895 as a familial clustering of colorectal and other types of cancer, and then re-described in 1971 by Henry Lynch, HNPCC is now defined molecularly as an *inherited,* cancer-predisposing syndrome secondary to a deleterious germline mutation in one of a group of DNA mismatch repair (*MMR*) genes.[1,2] The *MMR* genes correct sequence errors in DNA that result from faulty replication, and are an essential part of a post-replication DNA repair system. This genetic machinery is as complex as the genetic replication machinery itself, underscoring the importance of the *MMR* system.

An estimated 150,000 Americans may be carriers of the HNPCC mutation and have a 90% lifetime risk for developing some type of cancer. Up to 80% of carriers will develop colorectal cancer by the age of 70 years.

see Hereditary Colon Cancer page 10

Hereditary Colon Cancer

Continued from page 9

Up to 70% of female carriers are at risk for endometrial cancer by age 70. With germline mutation analysis, the aberrant genetic fingerprint is often detectable before the development of cancer. Unfortunately, our current capabilities have not advanced to the point of being able to use this information to repair the faulty gene and prevent the development of disease in affected individuals. However, we do possess the ability to reduce cancer risk in selected patients by removing susceptible organs before malignant transformation occurs.[3]

Understanding the Disease: The Pathologist

HNPCC, or Lynch Syndrome, is divided into Lynch I and Lynch II. Muir-Torre Syndrome and Colorectal Cancer Type X are also members of the HNPCC group. Muir-Torre is not commonly seen in the clinical setting. These diseases are often discovered by the pathologist postoperatively, on finding certain pathologic features during the examination of the resected surgical specimen.

In ***Lynch I***, colorectal cancer is the most common malignancy. The lifetime risk for a colorectal cancer in an individual with Lynch I syndrome is 80%. The average age at diagnosis is 44 years old, compared with 64 in the sporadic form of colon cancer. Multiple generations are usually affected. Most of the neoplasms are poorly differentiated and located proximal to the splenic flexure. Synchronous lesions occur in 7% of cases compared with 1% in sporadic cases. Metachronous lesions are found in 45% of patients with HNPCC compared with 5% of those with sporadic colon cancer, signaling a possible mismatch repair defect. Colorectal cancer occurring in the absence of a visible polyp or polyposis is the final phenotypic expression of the MMR mutation. It is important to note that a polyp, whether visible or occult, is the precursor lesion of the colorectal cancer.

Lynch II describes the association of colorectal cancer with extracolonic malignancies. In women with Lynch II, there is a 50% to 70% lifetime risk for endometrial cancer, with the average age at diagnosis being 46 years.[4] Other malignancies in Lynch II are ovarian cancer (3%-13%), gastric cancer (2%-13%), transitional cell carcinoma of the ureter and renal pelvis (1%-12%), small bowel cancer occurring most commonly in the duodenum and jejunum (4%-7%), central nervous system tumors, most often glioblastomas (1%-4%), and hepatobiliary cancer (2%).[5]

Muir-Torre Syndrome is a rare syndrome consisting of multiple benign and malignant neoplasms. It may be a variant of Lynch II. Sebaceous gland adenomas are the most common marker of the disease and are often found on the head. Keratoacanthomas, which may begin as a red nodule and progress to a shiny nodule with telangiectasia and a central crust, are located on the face and dorsum of the hands. Visceral carcinomas are frequent, occurring in one-half of patients with Muir-Torre. The colonic neoplasms are the most frequent malignancies found in Muir-Torre Syndrome and are usually located proximal to the splenic flexure. Genitourinary tumors are the second most common malignancy.[6]

Detection of the deleterious germline mutation has become the ultimate diagnostic criterion for HNPCC. The mutant gene is identified, as is the exact nucleotide mutation. This valuable information can be used in screening and in the genetic counseling of family members.

Familial Colorectal Cancer Type X is a disease in which affected patients meet the clinical criteria of the nonpolyposis syndrome but do not have the MMR defect found in that syndrome. More specifically, patients with Familial Colorectal Cancer Type X do not have a demonstrable mismatch repair mutation in one of the known *MMR* genes. The risk of developing colorectal cancer or extracolonic neoplasia is lower than that in patients with HNPCC, and the age at diagnosis of colon cancer is older than that in patients with Lynch Syndrome. Colonic malignancies are not predominantly right-sided as in patients with Lynch Syndrome. Unlike patients with the Lynch II syndrome, these individuals do not have a propensity to develop malignancies in other organ systems.[7]

Understanding the Genetics: The Geneticist

HNPCC is transmitted through germ cells in an autosomal dominant fashion and is highly penetrant. Germline cells are those cells passed down through generations. The commonly involved genes are *MSH2,* found in 60% of HNPCC mutations, and *MSH6,* found in 10% of mutations. Both are located on chromosome 2. *MLH1,* located on chromosome 3, is responsible for 30% of mutations. Numerous other genes account for rare cases of HNPCC. These genes normally produce proteins responsible for removing and repairing specific nucleotide sequences in DNA which may have become corrupted as a result of faulty replication. One copy of the mutant HNPCC gene is found in all cells and in all tissues of carriers. A second, normal copy of the gene from the unaffected parent is also present in all cells. Any event causing a mutation and inactivation of the normal gene in colorectal epithelium or other susceptible epithelium results in a transcriptional silencing of an important part of the MMR genetic machinery. This mutation is considered to be a "second hit," as both genes coding for the production of mismatch repair proteins become nonfunctional. Without mismatch repair, there is a rapid accumulation of somatic mutations and a neoplastic cascade leading to tumor development, which is the ultimate expression of the HNPCC phenotype.

The defect in *MMR* genes also leads to mutations in "bystander" genes, known as microsatellites. Microsatellites are short, non-coding, tandemly repeated DNA sequences of one to six nucleotide bases located primarily on the telomere or centromere, but also located next to the coding region of *MMR* genes. These sequences are unique to each individual. They can be affected by a mutation termed *microsatellite instability* (MSI). MSI results from the erroneous insertion, deletion or mis-incorporation of bases during DNA replication or recombination, with failure of the *MMR* system to correct these errors.[8] In HNPCC, mutant microsatellites begin to accumulate and can be detected in the tumor tissue of 95% of affected patients using fluorescent multiplex polymerase chain

see Hereditary Colon Cancer page 12

Features of Lynch I and Lynch II Forms of HNPCC

Lynch I

- Colorectal cancer is most common malignancy
- Lifetime risk for a colorectal cancer, 80%
- Average age at diagnosis, 44 years old
- Multiple generations usually affected
- Most neoplasms are poorly differentiated and located proximal to the splenic flexure
- Synchronous lesions occur in 7% of cases (compared with 1% in sporadic cases)
- Metachronous lesions found in 45% of patients with HNPCC (compared with 5% of those with sporadic colon cancer)
- Colorectal cancer in the absence of a visible polyp or polyposis is the final phenotypic expression of the *MMR* mutation.
- A polyp, visible or occult, is the precursor lesion of the colorectal cancer.

Lynch II

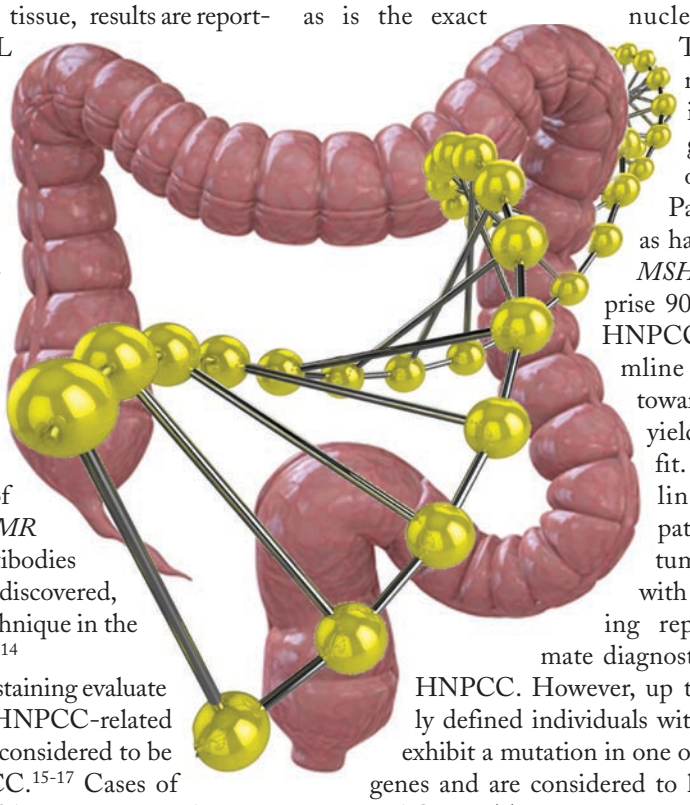
- Describes the association of colorectal cancer with extracolonic malignancies.
- In women, there is a 50% to 70% lifetime risk for endometrial cancer (average age at diagnosis, 46 years[4])
- Lifetime risk for ovarian cancer, 3%-13%
- Lifetime risk for gastric cancer, 2%-13%
- Lifetime risk for transitional cell carcinoma of the ureter and renal pelvis, 1%-12%
- Lifetime risk for duodenal or small bowel cancer, 4%-7%
- Lifetime risk for central nervous system tumors, most often glioblastomas, 1%-4%
- Lifetime risk for hepatobiliary cancer, 2%[5]

HNPCC, hereditary nonpolyposis colorectal cancer; **MMR**, mismatch repair

Hereditary Colon Cancer

Continued from page 10

reaction-capillary electrophoresis. The tumor microsatellite nucleotide repeats are compared with the repeats found in normal tissue adjacent to the tumor tissue. The tumor is considered to be microsatellite-unstable if the tumor repeats are different from the normal tissue repeats. MSI testing can be performed on fresh tissue or fixed paraffin blocks. In 1993, the genetics underlying mismatch repair were elucidated, allowing for MSI testing of tumor tissue in an attempt to diagnose HNPCC.[9-12] Depending on the number of abnormal nucleotide repeats found in the tumor tissue, results are reported as MSI-H (high), MSI-L (low) or MSI-S (stable).



An alternative detection technique consists of using antibodies to normal *MMR* gene proteins, combined with immunohistochemistry (IHC) fluorescent staining. IHC testing can be performed on fresh tissue or fixed paraffin blocks. Lack of staining is usually considered to be a positive test result, indicating loss of the normal protein product. This is due to the existence of a mutant, nonfunctioning *MMR* gene. In 1996, monoclonal antibodies to *MMR* gene proteins were discovered, allowing for this additional technique in the search for *MMR* mutations.[13,14]

Both MSI testing and IHC staining evaluate the phenotypic results of the HNPCC-related *MMR* gene mutation and are considered to be surrogate markers for HNPCC.[15-17] Cases of false-positive results and rare false–negative results exist and these must be considered as the clinician begins the evaluation. Higher detection sensitivities of up to 98% have been reported with use of MSI testing in combination with IHC, compared with either test by itself. Tumor testing has a high sensitivity, and a lack of tumor MSI (MSI-S or MSI-L) or normal IHC staining effectively rules out the possibility of classic Lynch I or Lynch II. No genetic mutation in a known MMR gene will be found on further testing. However, because these patients fulfill the clinical criteria for having an inherited colorectal cancer, these cases have been termed Familial Colorectal Cancer Type X, or "the other half of HNPCC."[18-19]

Additionally, positive results (MSI-H) do not guarantee that a germline mutation will be found. An important and not uncommon example of a false-positive test result that, if left undiscovered, could lead to expensive and time-consuming testing, caused by hypermethylation and subsequent transcription silencing of *MLH1*. This is thought to be the etiology of 15% of *sporadic* colorectal cancers. This is an *epigenetic* (non-mutational) change and means that although the underlying DNA morphology and sequence is normal, gene function is affected by a superimposed error, in this case, methylation of *MLH1*. MSI testing will be positive for microsatellite instability, but will not distinguish *sporadic* from *inherited* disease. The *BRAF* gene manufactures a *BRAF* protein that is involved in transmitting signals related to cell growth. A *BRAF* gene mutation is present in the majority of sporadic tumors with hypermethylation, but is not found in cases of HNPCC germline mutations. The combination of MSI testing, *MLH1* hypermethylation testing and *BRAF* mutational analysis can help distinguish sporadic colorectal cancer from HNPCC, and help avoid otherwise unnecessary genetic testing and resulting patient anxiety.

Germline analysis, with the discovery of a genetic mutation in one of the *MMR* genes, is an important step in diagnosing HNPCC. It is performed on samples of whole blood. However, germline analysis is not widely available for all patients. Detection of the deleterious germline mutation has become the ultimate diagnostic criterion for HNPCC. The mutant gene is identified, as is the exact nucleotide mutation. This valuable information can be used in screening and in genetic counseling of family members. Patients identified as having a mutation in *MSH2* or *MLH1* comprise 90% of patients with HNPCC. Therefore, germline testing directed toward these genes will yield the most benefit. Finding a germline mutation in patients with MSI-H tumors or in tumors with absent IHC staining represents the ultimate diagnostic confirmation of HNPCC. However, up to 50% of clinically defined individuals with HNPCC do not exhibit a mutation in one of the known *MMR* genes and are considered to have Familial Colorectal Cancer Type X.

To summarize the most common possible test results, in patients meeting the clinical criteria for having HNPCC (criteria to be discussed in part 2), who have a colorectal cancer that is MSI-S or MSI-L, or shows normal IHC staining, classic Lynch Syndrome is effectively ruled out and the patient is considered to have Familial Colorectal Cancer Type X. If the tumor is found to be MSI-H, it may be a Lynch I or Lynch II tumor. Germline testing will confirm or rule out the diagnosis of HNPCC. If a germline mutation is not found in an MSI-H tumor, it may represent a case of *sporadic* colorectal cancer secondary to hypermethylation of *MLH1*. Further testing for this possibility may be performed by testing for a combination of a *BRAF* mutation and *MLH1* hypermethylation, using a test kit that can specifically evaluate this possibility.

Finally, there are many families with several members who have colorectal cancer but who do not demonstrate an underlying genetic basis for the disease. All available genetic test results are normal and noninformative. Clearly not every mutant genes involved in the production of a colon malignancy has been identified. Until such time as the genetic basis for colorectal cancer is completely delineated, clinicians will have to rely upon clinical guidelines to begin the screening process.

Part 2 of this series will examine screening strategies and treatment recommendations for HNPCC.

References

1. Classics in oncology, 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. By Aldred Scott Warthin, 1913. *CA Cancer J Clin.* 1985;35:348-359.

2. Lynch HT, Krush AJ. Cancer Family "G" revisited: 1895-1970. *Cancer.* 1971;27:1505-1511.

3. Benns MV, Luk T, Scoggins CR. Surgical prophylaxis for inheritable malignant diseases: colorectal and gastric cancer. *Am Surg.* 2009;75:463-469.

4. Chung D, Rustgi AK. The hereditary nonpolyposis colorectal cancer syndrome: genetics and clinical implications. *Ann Intern Med.* 2003;138:560-570.

5. Vasen HF, Möslein G, Alonso A, et al. Guidelines for the clinical management of Lynch Syndrome (hereditary nonpolyposis cancer). *J Med Genet.* 2007:44:353-362.

6. Schwartz RA, Torre DP. The Muir-Torre syndrome: a 25-year retrospect. *J Am Acad Dermatol.* 1995;33:90-104.

7. Lindor NM, Rabe K, Petersen GM. Lower cancer incidence in Amsterdam-I criteria families without mismatch repair deficiency: familial colorectal cancer type X. *JAMA.* 2005;293:1979-1985.

8. Iyer RR, Pluciennik A, Burdett V, Modrich PL. DNA mismatch repair: functions and mechanisms. *Chem Rev.* 2006;106:302-323.

9. Fishel R, Lescoe MK, Rao MR, et al. The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. *Cell.* 1993;75:1027-1038.

10. Leach FS, Nicolaides NC, Papadopoulos N, et al. Mutations of mutS homolog in hereditary nonpolyposis colorectal cancer. *Cell.* 1993;75:1215-1225.

11. Lindblom A, Tannergård P, Werelius B, Nordenskjöld M. Genetic mapping of a second locus predisposing to hereditary nonpolyposis colon cancer. *Nat Genet.* 1993;75:279-282.

12. Ionov Y, Peinado MA, Malkhosyan S, et al. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. *Nature.* 1993;363:558-561.

13. Salahshor S, Koelble K, Rubio C, Lindblom A. Microsatellite instability and hMLH1 and hMSH2 expression analysis in familial and sporadic colorectal cancer. *Lab Invest.* 2001;81:535-541.

14. Søreide K. Molecular testing for microsatellite instability and DNA mismatch repair defects in hereditary and sporadic colorectal cancers—ready for prime time? *Tumour Biol.* 2007;28:290-300.

15. Lindor NM, Burgart LJ, Leontovich O. Immunohistochemistry versus microsatellite instability testing in phenotyping colorectal tumors. *J Clin Oncol.* 2002;20:1043-1048.

16. Hampel H, Stephens JA, Pukkala E, et al. Cancer risk in hereditary nonpolyposis colorectal cancer syndrome: later age of onset. *Gastroenterology.* 2005;129:415-421.

17. Mangold E, Pagenstecher C, Friedl W, et al. Tumours from MSH2 mutation carriers show loss of MSH2 expression but many tumours from MLH1 mutation carriers exhibit weak positive MLH1 staining. *J Pathol.* 2005;207:385-395.

18. Lindor N. Familial colorectal cancer type X: the other half of hereditary nonpolyposis colon cancer syndrome. *Surg Oncol Clin N Am.* 2009;18:637-645.

19. Aaltonen L, Johns L, Järvinen H, et al. Explaining the familial colorectal cancer risk associated with mismatch repair (MMR)–deficient and MMR-stable tumors. *Clin Cancer Res.* 2007;13:356-361.

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