As our understanding of hereditary colorectal cancers expands, so too does our knowledge of the basic genetic patterns underlying these inherited diseases. With the advent of precise molecular diagnostics, our ability to detect subclinical disease widens. The physician on the front lines should be able to appreciate the molecular and genetic patterns of hereditary polyposis and non-polyposis colorectal cancers and how these patterns contribute to diagnosis, prognosis and cure. This review will discuss both the framework for inherited genes and how a clinician may take advantage of this framework to secure a diagnosis of inherited colorectal cancer.

Molecular Basics

A nucleotide, is formed by the combination of a five carbon sugar, one or more phosphates and one of the purine or pyrimidine nucleobases guanine, adenine, cytosine, or thymine. A tri-nucleotide sequence, also known as a triplet or codon, codes for and produces a specific amino acid using processes called transcription and translation. A long sequence of codons with a start codon and a stop codon defines a single gene, which codes for many amino acids. These amino acids bond together to form a protein. Proteins play integral roles in cellular signaling, cellular division, and cell death or apoptosis. Each protein is considered to be the protein product of its parent gene.

The gene is a long-term storage area for the genetic code, or DNA, and all of the genes form a set of blueprints used by the body to control cellular structure and functioning. Many genes reside on a linear stretch of DNA and the entire length of these genes plus the intervening, non-coding portions form a chromosome.
Hereditary Colorectal Cancer Syndromes: Understanding Genetics

PRACTICAL APPROACHES TO THE DIAGNOSIS AND TREATMENT OF COLORECTAL CANCER #1

Table 1. Commonly Used Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
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<tr>
<td>HNPCC</td>
<td>Hereditary nonpolyposis colorectal cancer (Lynch Syndrome)</td>
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<tr>
<td>FAP</td>
<td>Familial Adenomatous Polyposis</td>
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<tr>
<td>AFAP</td>
<td>Attenuated Familial Adenomatous Polyposis</td>
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<tr>
<td>MMR</td>
<td>Mismatch repair - a gene implicated in the development of HNPCC</td>
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<tr>
<td>MLH</td>
<td>MutL Homolog - a gene commonly associated with HNPCC</td>
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<tr>
<td>MSH</td>
<td>MutS Homolog - a gene commonly associated with HNPCC</td>
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<tr>
<td>MSI</td>
<td>Microsatellite instability - short sequences of repeated DNA</td>
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<tr>
<td>IHC</td>
<td>Immunohistochemistry - A process of staining proteins within a cell</td>
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<tr>
<td>BRAF</td>
<td>Beta Rapidly Accelerated Fibrosarcoma - A protein affecting cell differentiation and division</td>
</tr>
<tr>
<td>APC</td>
<td>Adenomatous polyposis coli - a gene responsible for preventing uncontrolled growth of a cell</td>
</tr>
<tr>
<td>PTT</td>
<td>Protein truncation test (aka IVSP) - see text</td>
</tr>
<tr>
<td>IVSP</td>
<td>In-vitro synthesized protein assay (aka PTT) - see text</td>
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SPORADIC, FAMILIAL AND INHERITED DISEASE

Of all colon cancers, 80% to 90% occur sporadically, with no known etiology. 10% to 14% 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. Five percent of patients have an inherited or hereditary form of colon cancer and a causative genetic abnormality has been found to be associated with the malignancy.

HEREDITARY NONPOLYPOSIS COLORECTAL CANCER

Hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome accounts for four percent 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. MMR genes correct sequence errors in DNA that result from faulty replication. The MMR genes are part of a post-replication DNA repair system.

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 HNPCC mutations. Both are located on chromosome two. MLH1, located on chromosome three 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 corrupt as a result of faulty (continued on page 27)
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 this second normal gene in colorectal epithelium or other susceptible epithelium causes a transcription silencing of an important part of the MMR genetic machinery. The mutation is considered to be a “second hit” as both genes coding for the production of mismatch repair proteins are now non-functional. Without mismatch repair, there is a rapid accumulation of somatic mutations and a neoplastic cascade leading to tumor development.

The defect in mismatch repair genes also leads to mutations in “bystander” genes, known as microsatellites. Microsatellites are short, non-coding, repeated DNA sequences of one to six nucleotide bases located next to the coding region of MMR genes. These sequences are unique to each individual. A mutation in these microsatellite sequences is 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 mismatch repair system to correct these errors. In HNPCC, mutant microsatellites begin to accumulate and can be detected in the tumor tissue of 95% of affected patients. 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. In 1993, the genetics underlying mismatch repair were elucidated, allowing for MSI testing of tumor tissue in an attempt to diagnose HNPCC. 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. 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, non-functioning mismatch repair gene. In 1996, monoclonal antibodies to mismatch repair gene proteins were discovered, allowing for this additional technique in the search for MMR mutations.

Both MSI testing and IHC staining evaluate the phenotypic results of the HNPCC MMR gene mutation and are considered to be surrogate markers for HNPCC. Higher detection sensitivities of up to 98% have been reported when using MSI testing in combination with IHC, as compared to using either test by itself. Tumor testing has a high sensitivity, and a lack of tumor microsatellite instability (MSI-S or MSI-L) or normal IHC staining effectively rules out the possibility of having classic Lynch I or Lynch II. However, certain patients may meet the clinical criteria for having an inherited colorectal cancer but their genetic testing may be unrevealing. These patients will carry the diagnosis of Familial Colorectal Cancer Type X, or “the other half of HNPCC”.

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 which, if left undiscovered could lead to expensive and time consuming testing, is 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 sequence is normal, gene functioning 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 βRAF gene manufactures a βRAF protein, which is involved in transmitting signals related to cell growth. A βRAF 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 βRAF mutation analysis can help distinguish sporadic colorectal cancer from HNPCC and help to avoid otherwise unnecessary genetic testing and the resulting patient anxiety.

Germline analysis is performed on samples of whole blood and detection of a 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 HNPCC patients. Therefore, germline testing directed toward these genes will yield the most obvious benefit. However, up to 50% of clinically defined individuals with HNPCC...
do not display a mutation in one of the known MMR genes and are considered to have Familial Colorectal Cancer Type X.

The Investigation Begins

The initial step in the evaluation is to determine whether the patient or family member meets the Amsterdam-2 criteria, the Bethesda Guidelines or has a suspicious history which does not meet the strict clinical screening criteria. It is important to note however, that in patients who are undergoing genetic testing, specific genetic information may be uncovered. Patients must be given informed consent prior to undergoing this testing and genetic counseling must be available.

Following a thorough history and physical exam, further investigation depends on the presence or absence of tumor tissue available for molecular and genetic testing.

When Tumor Tissue is Available

When tumor tissue is available, it is first tested for microsatellite instability. This testing has a sensitivity of 98%, and any patient who is microsatellite instability-stable (MSI-S) or microsatellite instability-low (MSI-L) most likely does not have HNPCC. Further molecular testing of this group is unhelpful, with a low yield of any new information. However, this group of patients now carries a diagnosis of Familial Colorectal Cancer Type X, which references an inherited etiology as the origin of the tumor caused by an as of yet unknown genetic mutation. Patients in this group appear to have a lower overall incidence of colorectal cancer and lower risk for non-colorectal cancers than families with documented HNPCC.\(^9\) The colorectal cancers seem to occur at a later age as well. Surveillance in this group of patients is different than in patients with a diagnosis of HNPCC. It is started five or ten years earlier than the earliest diagnosed colorectal cancer in the family, and no less often than every five years.

Tumors that are microsatellite instability-high (MSI-H) are subjected to IHC staining, searching for the precise identification of the mutant gene. If IHC staining is normal, with normal protein expression of the known MMR genes, the test is considered non-informative. However, both the patient and family members are followed as if they carry a diagnosis of HNPCC. Even though the test is non-informative, emphasis is placed on intensive surveillance so as not to miss any patients in this group who might have HNPCC. The major drawback with this approach is that patients who in fact do not have HNPCC may undergo screening and continued intensive surveillance as if they are carrying the mutation. This has obvious psychological and financial costs as well as the downside of any morbidity associated with the surveillance procedures.

Patients or family members with absent protein staining by IHC undergo germline sequencing of the newly discovered mutant MMR gene. If germline testing confirms the mutation, this patient or family member is now identified as having HNPCC.

It should be noted that the above diagnostic scheme is one of several possible methods of investigation and that tests used should be tailored to the needs of each individual institution. Many hospitals will use IHC staining as the first diagnostic step while MSI testing is used only on a selective basis.

As MLH1 hypermethylation accounts for 20% of all sporadic colorectal cancers, tumors demonstrating high microsatellite instability and absent MLH1 staining on IHC, and in whom no MMR gene mutation is found on germline analysis will undergo testing looking for sporadic, non-inherited disease or an epigenetic mutation. Attention is focused on MLH1 because, with few exceptions, it is the methylated gene commonly associated with sporadic colorectal cancer. An epigenetic mutation is one in which the underlying DNA sequence is normal and a change, such as nucleotide hypermethylation has occurred on top of this normal DNA sequence. Although it affects gene function, this change is not considered to be inherited.

The Braf gene is involved in cell signaling. When the Braf gene mutates, cell growth and functioning is affected. Patients with absent protein staining for MLH1 who test positive for a Braf mutation are considered to have sporadic, non-inherited disease. Surveillance of this group and family members is the same as for the general population.

If the hypermethylation and Braf tests are negative, the genetic test results are considered to be non-informative and further surveillance is as for patients having HNPCC (figure 1).

When Tumor Tissue is Not Available

In patients meeting the clinical screening criteria or guidelines, who have no available tissue for study (the patient has previously undergone a tumor resection and the tissue is not available, or in family members of patients with known HNPCC), germline MMR
gene analysis is the first and only molecular evaluation performed.

If there is a family history of a known specific genetic mutation, this same known gene is sequenced in the patient. Testing for an already known mutant gene avoids having to laboriously analyze all MMR genes. In patients with no available tissue for study and in whom there is no available genetic information, analysis of all MMR genes is performed. If a mutation is found, a diagnosis of HNPCC is made and surveillance proceeds accordingly (figure 2).

FAMILIAL ADENOMATOUS POLYPOSIS

Familial adenomatous polyposis (FAP), an inherited disorder, is responsible for one percent of all colorectal malignancies. Caused by a mutation in a single gene, the Adenomatosis Polyposis Coli (APC) gene, FAP can lead to a radical change in the structure and functioning of the body.

In both FAP and sporadic colorectal cancers, a mutation of the APC gene is one of the earliest events leading to polyp formation and subsequent malignant degeneration. This is known as the adenoma-carcinoma sequence. The APC gene mutation has a high penetrance rate, meaning that individuals with the mutated gene (the genotype) will almost surely develop polyps (the phenotype).

80% of patients will have a family history of FAP or AFAP, with a known, precisely located mutation, heightening diagnostic suspicion and making the diagnosis of FAP straightforward. However, 20% of patients will have a de-novo mutation in an unknown gene location.

Attenuated Familial Adenomatous Polyposis

Attenuated Familial Adenomatous Polyposis (AFAP) is characterized by the formation of fewer, more proximal polyps developed at a later age. Clinically, AFAP has been recognized relatively recently. It may be a variant of FAP, or may be a disease in its own right.

Securing a diagnosis of AFAP is more challenging but must be considered in younger patients with between ten and 100 proximally located colonic polyps. The polyps are often flat. An upper gastrointestinal examination must be performed in patients with FAP or AFAP, as 80% to 90% will develop duodenal or periampullary adenomas. The polyps are commonly flat. They are diagnosed at an average age of 44 years. The carcinomas in AFAP develop at age 56 compared with FAP in which the average age at diagnosis is 10 or 15 years earlier. It is possible that the differential in age of onset of the polyposis and malignant transformation between FAP and AFAP is due to a lack of earlier recognition of AFAP by physicians and patients, rather than being a true difference in the age of onset.

It is often difficult to distinguish between FAP and AFAP based solely on the number of polyps seen on examination. In a single family with a single mutation, the number of colonic polyps in each family member may vary widely. In fact, there is evidence that AFAP and FAP may not be separate diseases, but different manifestations of a single entity. Extracolonic disease is
similar in both forms of polyposis. Genetically, FAP and AFAP are associated with a large number of different APC mutations. Clinically, patients with fewer than one hundred polyps may have FAP, AFAP or HNPCC. In a single family, patients may present with widely differing clinical manifestations.

Even though the polyps in AFAP are predominantly right sided and the mutation is usually located on either end of the APC gene, the underlying disease remains FAP. AFAP may simply be a form of FAP with mild expression.

**APC I1307K**

360,000 American Ashkenazi Jews are carriers of a mutant gene located on codon 1307 of the APC gene. This represents five percent of the Ashkenazi population. People carrying this mutation, known as APC I1307K, are at a 1.7 times greater risk of colorectal neoplasia compared with those who do not have this mutation. Additionally there are greater numbers of adenomas and colorectal cancers in this group, with a younger age at diagnosis. It is estimated that APC I1307K is responsible for up to four percent of all colorectal cancers in Ashkenazi Jews. Although the impact of this mutation is not fully understood at this time, it is thought that genetic testing of this entire population, irrespective of a family history of colorectal cancer, followed by appropriate clinical screening and surveillance might benefit the mutation carriers who are expected to develop colorectal cancer.

**From Genotype to Diagnosis**

As 96% of Adenomatous Polyposis Coli (APC) gene mutations in FAP result in a truncated protein product, the protein truncation test (PTT) has been developed to identify the area of the mutant APC gene. The APC gene mutation causes a premature termination of translation. Translation is the process whereby the nucleic code is translated into amino acids. The protein truncation test is also known as in vitro synthesized protein assay (IVSP).

The DNA to be tested is extracted from lymphocytes in whole blood. The PTT has been found to be successful in confirming a diagnosis in 85% of cases. Failure to diagnose FAP by PTT does not exclude the diagnosis and the clinician should not rely on a negative test result and falsely reassure the patient at this point. In those 15% of mutations missed by PTT, electrophoretic migration is used to find the undiscovered mutation. This sequential testing using two techniques has become commonplace and has a high sensitivity and high specificity (figure 3).

If testing is successful in identifying a mutant APC protein, the mutated nucleotides causing the truncation can be precisely identified with gene sequence analysis. This may provide valuable information as to both diagnosis and phenotypic expression of the disease. The mutational information can also be used for comparison with family members being tested for the same mutation (figure 4).

**An Educated Diagnosis**

The primary care provider must have a high suspicion for those patients meeting the Amsterdam or Bethesda criteria. A patient with a suspicious personal or family history can be promptly referred to a specialist where endoscopy, biopsy, genetic testing, definitive therapy, and further surveillance can be carried out. Alternatively, a primary care physician comfortable with endoscopy may wish to begin his or her own workup and refer to a specialist for further medical or surgical therapy. Most
importantly however, a negative genetic test or biopsy result should not deter early referral or management by a specialist.

A clinician who is knowledgeable in mutational genetics and who cares for patients at the early part of the diagnostic algorithm is in an ideal position to do a tremendous service for those patients and family members presenting with signs and symptoms suggestive of hereditary colorectal cancer.

With advances in early detection, and with the concomitant improvement in survival rates and functional outcomes, a confirmed diagnosis can be gratifying for the patient, the family, any newly diagnosed relatives, the astute physician who first considers and confirms the diagnosis, and the surgeon who performs the preventative or curative operative procedure. Further improvements in early detection will enhance our ability to diagnose and treat this family of genetic disorders.

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