The genes associated with each of the inherited syndromes of colon cancer have now been identified, and genetic testing is available for diagnosis. These syndromes include familial adenomatous polyposis, hereditary nonpolyposis colorectal cancer, Peutz–Jeghers syndrome, juvenile polyposis syndrome, and, possibly, Cowden's syndrome. Clinical genetic testing approaches have been developed for each of these syndromes and are now a part of accepted clinical care. Disease-causing mutations can be found in the majority of families affected with one of the inherited syndromes, and, most importantly, once a mutation is found in an index case of the family, relatives can be tested for the presence or absence of that mutation with near 100% accuracy. Cancer screening and management in syndrome families is then based on the results of genetic testing. For the physician to order and properly interpret genetic tests, a basic understanding of the types of mutations that lead to inherited disease and the methods for detecting them is vital. These issues will be presented. Additional clinical issues somewhat unique to genetic testing include genetic counseling and informed consent for genetic testing, both of which will also be reviewed. Often the most difficult aspect of genetic testing is deciding which patients and families should undergo the testing. Furthermore, this issue is quite specific for each of the syndromes. Thus, following presentation of general principles of selection for genetic testing, a detailed approach for identifying persons who should undergo testing for each of the individual syndromes will be given, together with relevant descriptions of the syndromes. Finally, the ongoing work to discover new and possibly more common but less penetrant colon cancer susceptibility genes that cause common familial colon cancer will be presented.

During the past decade the genetic etiology of all of the high-penetrance inherited colon cancer syndromes has been determined. Genetic testing to confirm syndrome diagnosis and to test asymptomatic relatives has become a part of standard care for persons and families with these conditions. Cellular mechanisms related to the involved genes are now being defined. This knowledge is likely to identify molecular targets for both cancer prevention and therapy. Present methods and approaches to clinical genetic testing will be the focus of this article, although the cellular and molecular pathways related to the disease genes will also briefly be described.

The overall approach to genetic testing is similar for all of the syndromes. A person with a clinical diagnosis of the condition in question first undergoes genetic testing to find the responsible mutation in the family. DNA for genetic testing is obtained from the white blood cells of a peripheral blood sample. The likelihood of finding the disease-causing mutation in this first or “index” case varies from 50% to over 90%, depending on the syndrome. However, finding the mutation is of substantial clinical utility. It first confirms the precise diagnosis in the index case and then allows testing in relatives. Testing in relatives, called “mutation specific testing,” is inexpensive compared with the initial test of the index case, and the accuracy is near 100% because it examines only for the presence or absence of the disease-causing mutation found in the index case. Screening and management can be based on the results of this testing.

Perhaps the most difficult aspect of genetic testing for clinicians is knowing when to use genetic testing and whom to test. This decision can be straightforward when dealing with one of the polyposis conditions because they are each characterized by a rather distinct phenotype. A person with a fully developed polyposis syndrome can usually be diagnosed clinically and thus can serve as an index case in a family for initial genetic testing. However, when the first person in a family being considered for testing does not have a fully developed syndrome, and the family history is not clear, the decision to test can be difficult. Guidelines as to when to proceed with genetic testing in this situation will be presented.

Abbreviations used in this paper: AFAP, attenuated FAP; FAP, familial adenomatous polyposis; HNPCC, hereditary nonpolyposis colorectal cancer; MAP, MYH-associated polyposis; PTT, protein truncation test.
However, the most difficult situation in which one must decide on genetic testing involves the syndrome of hereditary nonpolyposis colorectal cancer (HNPPC). There is no distinctive individual phenotype for HNPPC. For clinical diagnosis, one must rely on family history and age of cancer diagnoses. However, the family history of cancer is often inaccurate or incomplete.\textsuperscript{9–12} Furthermore, many families are small, and genetic predispositions are not fully expressed. Overuse of genetic testing is expensive and may lead to unnecessary concern among family members, but under use of testing may leave an HNPPC family without a definitive diagnosis and with no way to decide which family members should have special screening.

It is estimated that 10% to 15% of patients with colon cancer should be considered for genetic testing to find the approximate 3% to 5% that will have HNPPC. In one study, 19% of sequential colon cancer patients met Bethesda guidelines (discussed below) and therefore should have been considered for genetic screening or testing, although only a tiny fraction of those actually were tested.\textsuperscript{11} Also, as many as 0.5% to 1.0% of adults presenting for colon cancer screening should similarly be considered for testing. How should a clinician decide which persons and families should have genetic testing for HNPPC? Fortunately, a number of helpful and efficient approaches have now been developed to determine when genetic testing should be recommended for the diagnosis of this condition. These will be presented.

In this review the syndromes and genes relevant to inherited colon cancer will first be briefly outlined. The types of DNA mutations that give rise to inherited colon cancer, and the genetic methodologies to detect these mutations will then be summarized. The general approach to genetic testing will then be presented, together with notes on genetic counseling and informed consent. Finally, each of the inherited syndromes of colon cancer will be described in detail, together with genetic testing approaches specific to each syndrome. The article will end with a brief review of the present gene discovery work addressing the less penetrant but more common category of familial colon cancer outside the known inherited syndromes.

### Colon Cancer Syndromes and Their Associated Genes

#### The Syndromes

A number of inherited syndromes give rise to an increased risk of colorectal cancer. Traditionally, these syndromes have been divided according to the pathology of the intestinal polyps that occur. There are syndromes that give rise to adenomatous polyps and those in which hamartomatous polyps are found.\textsuperscript{2,6–8,13} See Table 1 for a summary of these syndromes and their associated genes. The adenomatous polyp syndromes include familial adenomatous polyposis (FAP) and its subtypes Gardner syndrome, Turcot syndrome, and attenuated FAP and HNPPC with its subtypes Turcot syndrome (some families) and Muir–Torre syndrome. The hamartomatous polyposis syndromes include Peutz–Jeghers syndrome, juvenile polyposis syndrome, and Cowden's syndrome with its related subtype Bannayan–Ruvalcaba–Riley syndrome.

All of the syndromes have a very high risk of colon cancer, except possibly Cowden's syndrome, in which the risk of colon cancer is debated. FAP accounts for less than 1% of colon cancer cases, and HNPPC accounts for 3% to 5%.\textsuperscript{3,6,14} The hamartomatous polyposis syndromes are all very rare and together account for substantially less than 1% of colon cancer cases. However, despite the relatively unusual nature of all of these syndromes, they are important because of their extreme cancer risks. They are also important because cancer, especially colon cancer, can be prevented in most cases with proper syndrome recognition and screening. Furthermore, genetic testing is needed for diagnosis with sufficient frequency that all clinicians should have some familiarity with the conditions and with the indications for genetic testing.

#### The Genes

The genes associated with each of the colon cancer syndromes have now been identified (Table 1). Mutations in these genes give rise to the syndromes. All the syndromes are autosomal dominantly inherited, except for the rare case of FAP caused by MYH mutations.\textsuperscript{15} This condition is recessively inherited, is now called MYH-

### Table 1. Inherited Colon Cancer Syndromes and Their Associated Genes

<table>
<thead>
<tr>
<th>Syndromes</th>
<th>Associated genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syndromes with adenomatous polyps</td>
<td></td>
</tr>
<tr>
<td>Familial adenomatous polyposis</td>
<td>APC, MYH (rare)</td>
</tr>
<tr>
<td>(FAP), includes Gardner syndrome,</td>
<td></td>
</tr>
<tr>
<td>two thirds of Turcot syndrome, and</td>
<td></td>
</tr>
<tr>
<td>attenuated FAP</td>
<td></td>
</tr>
<tr>
<td>Hereditary nonpolyposis colorectal</td>
<td>Mismatch repair genes:</td>
</tr>
<tr>
<td>cancer (HNPPC), includes one</td>
<td>MLH1, MSH2; MSH6 (uncommon),</td>
</tr>
<tr>
<td>third of Turcot syndrome and</td>
<td>PMS2 (rare)</td>
</tr>
<tr>
<td>Muir–Torre syndrome</td>
<td></td>
</tr>
<tr>
<td>Syndromes with hamartomatous polyps</td>
<td></td>
</tr>
<tr>
<td>Peutz–Jeghers syndrome</td>
<td>STK11 (LKB1)</td>
</tr>
<tr>
<td>Juvenile polyposis syndrome</td>
<td>MADH4, BMPR1A</td>
</tr>
<tr>
<td>Cowden’s syndrome, includes</td>
<td>PTEN</td>
</tr>
<tr>
<td>Bannayan–Ruvalcaba–Riley (BRR)</td>
<td></td>
</tr>
<tr>
<td>syndrome</td>
<td></td>
</tr>
</tbody>
</table>
associated polyposis (MAP), and will be discussed further under FAP.

Identification of the genes associated with the syndromes has allowed a more precise classification of the syndromes. For example, mutations of the same gene can give rise to similar but historically somewhat different phenotypical syndromes, such as FAP and Gardner syndrome. Both of these arise from mutations of the adenomatous polyposis coli (APC) gene but are now considered variations of the same disease. Several of the syndromes can also be caused by mutations of different genes. FAP can be caused by mutations of both the APC and the MYH genes. These 2 genes exhibit quite different disease mechanisms but result in a similar phenotype. HNPCC is caused by mutations in any one of several different genes. However, in this instance, the protein products of the different genes are all involved in the same DNA repair mechanism, called mutation mismatch repair. Similarly, the 2 genes associated with juvenile polyposis syndrome, MADH4 (SMAD4) and BMPR1A are both components of the signaling pathway for TGF-β and the bone morphogenetic proteins (BMPs).

Procedure for Obtaining DNA

DNA for genetic testing is derived from the white blood cells of a peripheral blood sample. Many clinical pathology laboratories can process the blood to DNA for shipping to a genetic testing facility. Even if such processing is not locally available, testing laboratories will supply a kit for sending the blood directly. Testing laboratories can be found at www.genetests.org.

As the mutations being looked for are germ line or inherited DNA mutations, they are present in every cell of the body and thus in the circulating white blood cells. This is unlike many of the mutations that are found in a tumor itself. They are often somatic or acquired mutations, only occurring in the tumor tissue. Such mutations are part of the carcinogenesis process but are usually not helpful for the diagnosis of an inherited susceptibility syndrome. It is thus of critical importance to distinguish between somatic mutations and germ line, hereditary mutations, especially for interpreting molecular marker results.

Mutations and Mutation Detection Methods

Types of DNA Mutations

There are many types of genetic mutations that can result in a defective protein or decreased protein expression and thereby cause inherited disease. For clarity, the specific type of mutation is often reported as part of the genetic testing results. Thus, familiarity with the various mutation types is essential to understanding results of clinical genetic testing.

It is also important to know that most DNA mutations or alterations do not result in disease but are part of normal genetic variation. These alterations are called “polymorphisms” and are frequently observed. Polymorphisms can be completely inconsequential or can give rise to modest disease predisposition. A genetic testing laboratory first identifies mutations and then distinguishes those that cause disease from harmless mutations. However, if the functional result of a mutation cannot be determined, the report will indicate “sequence variation of uncertain significance.” Such a result is said to be uninformative, and clinical management must then rely on clinical findings alone. The various types of DNA mutations or alterations and how they relate to disease are outlined next.

Single DNA Base Changes

Proteins are made up of a specific sequence of amino acids, which is determined by the DNA triplet code. Each 3 DNA bases (also called DNA nucleotides) form a triplet code or “codon” that is specific for an amino acid. A single nucleotide change can have 1 of 3 results on a codon: (1) the specified amino acid may be the same because some amino acids have several different codons; (2) a different amino acid may be specified; or (3) a stop signal, called a stop codon, may be specified.

If the amino acid is not changed, the mutation is said to be silent. If a single amino acid is changed, the mutation is called a “missense” mutation. The effect of a single amino acid change or missense mutation is difficult to predict. The protein function may be unchanged, moderately changed, or severely changed. If the laboratory cannot determine the effect of the change, the report will likely read “sequence variation of uncertain significance.” Laboratories keep track of such changes. If the effect of the missense mutation later becomes apparent through laboratory or clinical observations, the ordering physician will be notified.

The third type of change possible from a single nucleotide alteration is the creation of a “stop” signal. A normal stop signal occurs at the end of each gene and appropriately terminates protein formation. There are 3 different triplet codes that cause a stop. If one of these is accidentally created by mutation, then protein translation is prematurely stopped and protein formation is truncated. This type of mutation is virtually always severe and disease causing. It is called a “nonsense” mutation.
Deletions and Insertions

Deletions and insertions are the second type of mutation. One or more nucleotide(s) are deleted from or added to the DNA sequence in this type of mutation. With insertions or deletions, the triplet reading sequence is shifted out of phase or “out of frame” so that a nonsense triplet code results. These mutations are called “frameshift” mutations. Frameshift mutations lead to erroneous amino acids and usually also to an accidental stop signal or “stop codon,” “downstream” from the mutation, also causing a shortened or truncated protein. Thus, deletions and insertions are virtually always disease causing, unless they consist of a multiple of 3 nucleotides. In that case, only amino acid changes occur, with potential results as described previously.

Splice Site and Regulatory Mutations

Genes consist of exons, which are the segments of the gene eventually translated into protein, and introns, which are intervening stretches of DNA that are spaced before, after, and between exons. Intronic nucleotide sequences immediately next to the exons determine how the gene is spliced and read. Mutations in these areas often have severe, disease-causing results because large segments of the gene are not translated into protein. Other mutations in areas of the DNA called regulatory segments are often very difficult to identify but may result in proteins not being expressed properly.

Large Deletions, Duplications, Translocations, and Inversions

Alterations in large segments of DNA fall into the categories of large deletions, duplications, and rearrangements (translocations and inversions) and are caused by a number of different genetic mechanisms. These types of errors almost always result in abnormal gene expression and disease.

Identification of Disease-Causing Mutations

A number of different laboratory methods are used to detect the various mutation types and determine whether they give rise to disease. It is important to know which methods are being used and what other methods are available if a suspected mutation is not initially found. DNA sequencing is the standard method for initial identification of mutations. However, there are several laboratory methods, often applied before sequencing, that indicate that a mutation is present and, at the same time, narrow the area of the gene at which the DNA sequencing must be done. These methods include conformation-specific gel electrophoresis, single-strand conformation polymorphism, and denaturing gradient gel electrophoresis, each of which will be described below.

Some types of mutations are not found by sequencing. These include large deletions and rearrangements. Several methods are used to detect these mutations and will likewise be described. Another method that will be described is one that has been developed to identify specifically the mutations that result in protein truncation, appropriately called protein truncation testing. The final testing method to be described is linkage testing by which DNA markers are used to indicate the presence of a mutation in members of a family, but the mutation itself is not identified.

Inherited conditions usually arise from a mutation of a single gene, although different families with the same disorder usually have separate and distinct mutations of that gene. An identical mutation identified in many seemingly separate families is usually a “founder” mutation, ie, one coming from a common ancestor. In some populations, founder mutations may account for a substantial fraction of the syndrome families in that population.

The success of identifying the causative mutation in an index case, ie, a person in the family clinically known to have the disease, varies depending on the disease. For the colon cancer syndromes, the success rates are given in Table 2. However, once a mutation is found in the index case, other family members can be tested for the presence or absence of that specific mutation, with a near 100% accuracy. This testing is called mutation-specific testing, and a number of methods can be used in this regard.

Once a clearly disease-causing mutation is found in an individual, clinical screening and management can then be guided by genetic diagnosis. If a disease-causing mutation cannot be found in the index case, however, then genetic testing is considered uninformative in the family, and all family members must be considered at risk for the disease. Alternatively, linkage analysis can

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Approximate success of finding a disease-causing mutation in the index case (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Familial adenomatous polyposis</td>
<td>80–90</td>
</tr>
<tr>
<td>Hereditary nonpolyposis colorectal cancer</td>
<td>50–70</td>
</tr>
<tr>
<td>Peutz–Jeghers syndrome</td>
<td>30–70</td>
</tr>
<tr>
<td>Juvenile polyposis syndrome</td>
<td>40–60</td>
</tr>
<tr>
<td>Cowden’s syndrome</td>
<td>80–90</td>
</tr>
</tbody>
</table>

NOTE. Sources are references 1, 2, and 8.
sometimes be used to indicate the likelihood that individual family members carry the mutant gene.

**Sequencing**

DNA sequencing is a standard method used to detect mutations. Sequencing is the process of determining the order of nucleotide bases (A, C, G, and T) for all the coding regions (exons) and certain intronic regions of the gene of interest. Although sequencing is the gold standard for clinical genetic testing, there are limitations, including cost and failure to detect some genetic rearrangements, large deletions, and also mutations in regulatory regions.

Sequencing can often be more efficiently done when complemented with an initial DNA screening method that both detects the presence of a mutation and also narrows the DNA area to be sequenced. The next 3 methods are such screening methods. Once one of these indicates a DNA variant, sequencing is then required to identify and confirm the precise mutation and determine whether it is a normal variant or a disease-causing mutation.

**Conformation-Specific Gel Electrophoresis**

Conformation-specific gel electrophoresis (CSGE), also called heteroduplex analysis (HA), is one method used to complement sequencing. A specific region of genomic DNA from 150 to 300 base pairs in length is amplified by the polymerase chain reaction (PCR) and is then denatured (which separates strands of DNA) and renatured (DNA strands anneal to their complementary strands). If there is a sequence variation (a mutation) in 1 of the 2 alleles, some strands will renature as a hybrid between the wild-type and the variant DNA strands, called a heteroduplex. When analyzed by gel electrophoresis, the hybrid DNA will migrate through the gel matrix at a different rate and can therefore be visualized as a distinct band on the gel. CSGE detects greater than 90% of mutations present.

**Single-Strand Conformation Polymorphism**

PCR amplification of a specific region of DNA is also the first step for single-strand conformation polymorphism (SSCP) analysis. The resulting double-stranded DNA is then denatured and quickly renatured so that some strands are not able to find their complementary strand before renaturation. A population of single-stranded DNA folded upon itself results, forming a secondary structure. The renatured DNA is next run on a gel, and DNA variants that are different from the normal sequence migrate at a different rate within the gel matrix, thus indicating the presence of a DNA variant or mutation. The success of this method to detect the presence of a mutation is 60%–95%.

**Denaturing Gradient Gel Electrophoresis**

Denaturing gradient gel electrophoresis (DGGE) is similar to CSGE, but differs in that the double-stranded DNA is run through a gel matrix that has an increasing gradient of urea and formaldehyde, chemicals that denature DNA. Variations or mutations in the DNA sequence will denature at different substrate concentrations and consequently migrate differently in the gel matrix compared with the wild-type DNA. DGGE can detect up to 95% of sequence changes.

**Methods for Detecting Large Deletions and Rearrangements**

Southern blotting is a commonly used method for the detection of large intragenic deletions, changes sufficiently large to go undetected by sequencing. The exons of a gene are interrogated with primers, and a gel electrophoresis is performed. Differing amounts of DNA between the 2 alleles (or parts of alleles) of the same exon indicate the presence of an intragenic deletion in 1 allele. A second, increasingly used method to detect intragenic deletions is quantitative PCR. In this method, PCR is performed on exons or sections of the gene. The observation of different amounts of DNA from a single section indicates a difference in sizes between alleles and thus a deletion in 1 allele.

Some deletions are sufficiently large enough so that they can be detected by karyotype analysis looking directly at chromosome preparations. In karyotype analysis, giemsa-banding patterns of metaphase chromosomes are examined for correct number and size of the chromosomes. In a related method, chromosomes can be probed and examined with fluorescent DNA markers usually specific to certain genes (fluorescent in situ hybridization or FISH). Rearrangements can be particularly difficult to detect and, depending on size, can escape detection by sequencing or the other methods described. Karyotype and/or FISH analysis can sometimes detect large rearrangements.

**Protein Truncation Test**

With the protein truncation test (PTT) method, protein is synthesized in vitro from DNA. If there is a nonsense mutation, ie, one that causes truncation of the protein, 2 bands are observed on the electrophoresis gel, 1 for the normal protein and 1 for the shortened protein. However, PTT fails to detect changes if there is a large deletion or if the truncation is at the very start or the very
end of the gene or gene segment being examined. Additionally, any mutations other than nonsense or truncating mutations cannot be detected. PTT is thus used to indicate the presence of a truncating mutation and, if positive, can be followed by sequencing to determine the exact mutation. The advantage of PTT is that mutations detected are always disease causing, but the disadvantage is that missense mutations and larger mutations are not detected.

**Linkage Analysis**

In linkage analysis, DNA markers near or within the gene in question are used to see whether the markers correlate with the disease phenotype in a family. Multiple family members known to have the disease are needed for the linkage testing to be effective. Sufficient numbers of DNA markers are now available so that linkage analysis can be effective in 90% to 95% of families if DNA is available from multiple affected family members, but, because the exact mutations are not being identified, asymptomatic members that are tested are given a likelihood that they carry a deleterious mutation. Likelihoods vary according to the markers available and the family structure but are not very helpful clinically unless they are >95% or <5%.

**Site-Specific Mutation Analysis**

Once a specific mutation is identified in an index case of a family, “site specific mutation analysis” also called “mutation specific analysis” is used to determine the presence or absence of that particular mutation in other family members. There are a number of PCR-based methods used, often specific to the mutation found in the family. The various techniques will not be described here, except to note that these techniques are all highly accurate and much less expensive than the original mutation identification in the index case of the family. In fact, accuracy is near 100% so that clinical screening and management of tested individuals can be based on the results. This accuracy is why genetic testing is so useful in a syndrome family. Thus, even though mutations cannot be identified in all index cases, once a mutation is identified in the index case, other family members can receive a relatively inexpensive genetic diagnosis with near 100% accuracy, both positive and negative.

**General Approach to Genetic Testing**

**Indications for Genetic Testing**

The American Society of Clinical Oncology recommends that genetic testing be offered when (1) the individual has personal or family history features suggestive of a genetic cancer susceptibility condition, (2) the test can be adequately interpreted, and (3) the results will aid in diagnosis or influence the medical or surgical management of the patient or family members at hereditary risk of cancer.

Assuming these general guidelines are met, there are 3 specific clinical situations in which genetic testing for the inherited syndromes of colon cancer should be considered: (1) when a syndrome diagnosis is already apparent clinically, (2) in relatives of a person with a known genetic diagnosis, and (3) when a syndrome is suspected but not certain on clinical grounds.

In the first instance, when an inherited cancer syndrome is apparent clinically, genetic testing is done to identify the disease-causing mutation in the relevant gene. Finding the mutation in this situation confirms the diagnosis and allows other family members to be tested with a high degree of accuracy as outlined previously. Failure to find a disease-causing mutation in this person known to have the inherited condition, ie, in the index case of the family, however, does not rule out the syndrome. As outlined above, there are a number of technical reasons why mutations cannot initially always be found. The genetic test result in this instance is said to be uninformative or nonspecific. Further genetic testing in relatives of the index case would therefore not be useful or even possible. Without the possibility of genetic testing, all family members who may have inherited the gene mutation responsible for the syndrome must still be considered at risk for the condition.

Genetic testing in relatives of an index case who was found to have a disease-causing mutation is then the second situation for genetic testing. Testing is done by mutation-specific testing methods as described in the previous section. This testing is near 100% accurate so that screening and management in relatives can be based on the results.

The third category of genetic testing is in persons suspected of having an inherited syndrome but the diagnosis remains uncertain on clinical grounds alone. Finding a disease-causing mutation in this setting confirms the diagnosis, but failure to find one, again, does not rule it out. The clinician is then left with clinically based judgment as to how likely the inherited condition may be and also how to manage that patient and the relatives, based on that likelihood.

There are a number of clinical findings that lead one to suspect an inherited colon cancer syndrome, but the diagnosis may not be certain. One should consider genetic testing in these situations as follows:
Table 3. Areas Covered in a Genetic Counseling Visit

<table>
<thead>
<tr>
<th>Collection of background information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient demographic data and relevant medical history</td>
</tr>
<tr>
<td>Family history with construction of a pedigree</td>
</tr>
<tr>
<td>Verification of family cancers, polyps, or other condition under evaluation</td>
</tr>
<tr>
<td>Patient’s perception of risk</td>
</tr>
<tr>
<td>Psychological stability and concerns</td>
</tr>
<tr>
<td>Patient educational issues covered</td>
</tr>
<tr>
<td>Basic concepts of inheritance</td>
</tr>
<tr>
<td>Characteristics of the syndrome in question, including age-specific risks</td>
</tr>
<tr>
<td>Syndrome management, including recommended approaches for prevention, if available</td>
</tr>
<tr>
<td>Determinations made</td>
</tr>
<tr>
<td>Likelihood of syndrome diagnosis</td>
</tr>
<tr>
<td>Estimated risks for self and family members</td>
</tr>
<tr>
<td>Optimal screening and prevention strategies</td>
</tr>
<tr>
<td>Utility of possible genetic testing</td>
</tr>
<tr>
<td>Informed consent</td>
</tr>
</tbody>
</table>

1. for FAP or attenuated FAP (including MAP) when 10 or more colonic adenomatous polyps have been found in a patient at 1 examination or over time;
2. for HNPCC when the Amsterdam criteria or revised Bethesda guidelines have been met (see below);
3. for Peutz–Jeghers syndrome when any Peutz–Jeghers polyps or typical peri-oral pigmentation are found;
4. for juvenile polyposis syndrome when 3 or more juvenile polyps have occurred; and
5. for Cowden’s syndrome when features of the syndrome are present.

Genetic Counseling, Informed Consent, and Cost of Genetic Testing

Genetic testing often provides a disease diagnosis long before any disease manifestations have become apparent and possibly even before disease screening is necessary. Thus, there may be substantial impact on family members who are being tested. Issues include psychologic impact, effects on family dynamics, and insurability questions. The patient and physician must understand these issues and be familiar with the benefits and risks of genetic testing before it is done. General guidelines for the appropriate use of genetic testing in any situation have been outlined, as well as guidelines specific to the colon cancer syndromes, which will be presented in more detail below. A genetic counseling visit can be of great assistance to both the physician and the patient considering genetic testing. The areas covered in a genetic counseling session are given in Table 3.

Informed consent is a necessary part of the patient educational process for genetic testing and is required by most laboratories that perform testing. The areas of discussion for the informed consent process are outlined in Table 4.

Genetic testing costs vary depending on the type of genetic testing done and the laboratory used for the testing. Laboratories offering testing are found at www.genetests.org. Some tests are clustered, usually resulting in cost savings. For example, some laboratories will do MYH testing when APC testing is ordered for the diagnosis of FAP, but APC mutations are not found. Additionally, as part of the testing package some laboratories will perform Southern blotting and/or quantitative PCR to detect large intragenic deletions and rearrangements if sequencing is unrevealing. Costs of genetic testing in the colon cancer syndromes are given in Table 5.

Specific Syndromes

Familial Adenomatous Polyposis

The disease and clinical management. FAP is an autosomal dominantly inherited disease with a prevalence of approximately 1 in 10,000 persons (Table 6). It accounts for less than 0.5% of colon cancer cases but is important because of the extreme colon cancer risk in affected patients. The most prominent feature of FAP is the emergence of hundreds to thousands of colonic adenomatous polyps, beginning at an early age and resulting in a near 100% risk of colon cancer if the colon is not removed. The average age of polyp development is 16 years and of colon malignancy is 39 years. The strict definition of FAP requires that 100 or more colonic adenomatous polyps be present, although many fewer polyps are obviously observed in younger FAP patients. Gastric, duodenal, and small bowel polyps also occur, but the risk of cancer in these areas as well as several other cancers is low compared with the colon (Table 6).

Variants of FAP include Gardner syndrome, which is characterized by the intestinal findings of FAP together with benign soft tissue and boney tumors, called epidermoid cysts and osteomas, respectively; Turcot syndrome, defined by CNS tumors (usually medulloblastomas) accompanying the colonic polyposis; and attenuated FAP (AFAP), in which the number of colonic polyps is extremely variable, but averages approximately 30, and the emergence of polyps and cancer is delayed by approximately 10 years. Desmoid tumors (benign connective tissue tumors) occur in approximately 20% of those with FAP and can result in significant morbidity when they are intra-abdominal and compress abdominal organs.

Approximately 20% to 30% of FAP patients without a known family history of the disease appear to represent
“new mutations.”22,25,26 Thus, parents and siblings are not found to have the disease on investigation, but children of the affected person are nonetheless at a 50% risk of the condition. This is unlike HNPCC in which new mutations are rarely verified. Additionally, biallelic MYH mutations may also occur in the setting of no family history and should be considered in these situations. However, with MYH mutations, as with any recessive disease, the siblings of an affected individual are at 25% risk for carrying biallelic mutations but not the children.

Clinical management includes genetic testing and colonic screening as outlined in Table 7 and periodic upper GI screening.2,6,27 A timely subtotal colectomy, restorative proctocolectomy or colectomy with mucosal proc-

### Table 4. Informed Consent for Genetic Testing

| Medical issues. The patient should understand |
| Details of the syndrome in question, including inheritance, cancer risks, screening guidelines and disease management |
| That medical care may be better directed with genetic diagnosis |
| That compliance with surveillance guidelines is necessary for benefit |
| That prevention may be improved with knowledge and compliance |

| Genetic issues. The patient should understand |
| The interpretation and implications of a positive, a negative, and an indeterminate genetic test result |
| That the cancer risk to self and family members can be better defined if genetic testing is successful |
| The methods of testing and the accuracy associated with testing options |
| The alternatives to genetic testing |

| Psychologic issues. The patient should understand that |
| Failure to detect a mutation in the first person tested in family may lead to frustration, anxiety, disappointment, or possible relief |
| A positive test may lead to anger, anxiety, guilt, stress, or self-image issues |
| A negative test in a member belonging to a family with a known mutation may lead to relief, decreased worry for self and offspring, or survivor guilt |

| Social/economic issues. The patient should understand that |
| Failure to detect a mutation in the first person tested in family results in no added information for self, children, and other at-risk relatives |
| A positive test may lead to possible insurance, employment, and social discrimination |
| A negative test in a member belonging to a family with a known mutation may lead to possible resolution of insurance problems, but family relationships may be positively or negatively affected |

| Special issues for testing in children. Parents and children (depending on age) should understand that |
| Children’s rights must be protected, despite the child’s ability to understand |
| Positive genetic testing may lead to self-image problems |
| Positive or negative results may lead to problems with parent-child relationships or sibling-sibling relationships |
| Positive results may lead to social stigmatization |

| Patients should understand the cost of testing, whether insurance coverage is present, and that workplace genetic discrimination is a possibility |
| Patients should understand that genetic testing results are completely confidential and will not be shared with family members unless they decide to do so themselves. |

### Table 5. Costs for Genetic Testing

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Laboratory method</th>
<th>Costs</th>
<th>Availability</th>
</tr>
</thead>
<tbody>
<tr>
<td>HNPCC</td>
<td>Linkage</td>
<td>$300 per person</td>
<td>Clinical</td>
</tr>
<tr>
<td>MSI testing (may include IHC)</td>
<td>$300–$500</td>
<td>Clinical</td>
<td></td>
</tr>
<tr>
<td>Protein truncation testing</td>
<td>$750</td>
<td>Clinical</td>
<td></td>
</tr>
<tr>
<td>Sequencing both MLH1 and MSH2 genes</td>
<td>$1200–$2400</td>
<td>Clinical</td>
<td></td>
</tr>
<tr>
<td>Sequencing MLH1, MSH2, MSH6</td>
<td>$2340–$2900</td>
<td>Clinical</td>
<td></td>
</tr>
<tr>
<td>Known mutation in family</td>
<td>$200–$500</td>
<td>Clinical</td>
<td></td>
</tr>
<tr>
<td>FAP</td>
<td>Linkage</td>
<td>$630–$5300 for up to 10 family members</td>
<td>Clinical</td>
</tr>
<tr>
<td>Protein truncation testing</td>
<td>$500–$1081</td>
<td>Clinical</td>
<td></td>
</tr>
<tr>
<td>Sequencing</td>
<td>$1650</td>
<td>Clinical</td>
<td></td>
</tr>
<tr>
<td>Known mutation in family</td>
<td>$285–$350</td>
<td>Clinical</td>
<td></td>
</tr>
<tr>
<td>Cowden’s syndrome</td>
<td>Sequencing</td>
<td>$775–$1400</td>
<td>Clinical</td>
</tr>
<tr>
<td>Known mutation in family</td>
<td>$200–$350</td>
<td>Clinical</td>
<td></td>
</tr>
<tr>
<td>Peutz–Jeghers syndrome</td>
<td>Sequencing</td>
<td>$775–$1400</td>
<td>Clinical</td>
</tr>
<tr>
<td>Known mutation in family</td>
<td>$200–$350</td>
<td>Clinical</td>
<td></td>
</tr>
<tr>
<td>Juvenile polyposis syndrome</td>
<td>Sequencing</td>
<td>$1625</td>
<td>Clinical</td>
</tr>
<tr>
<td>Known mutation in family</td>
<td>$200</td>
<td>Clinical</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Information assembled by Rebecca Hulinski, MS, genetic counselor, Huntsman Cancer Institute, from personal laboratory contacts and laboratory sources identified on www.genetests.org.

HNPCC, hereditary nonpolyposis colorectal cancer; MSI, microsatellite instability; IHC, immunohistochemistry; FAP, familial adenomatous polyposis.
Table 6. Clinical Features of the Inherited GI Syndromes That Predispose to Cancer

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Relevant gene</th>
<th>Polyp histology</th>
<th>Polyp distribution and frequency</th>
<th>Mean age of GI symptom onset</th>
<th>CRC risk (mean age of diagnosis)</th>
<th>Most prominent extra colonic features</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAP</td>
<td>APC</td>
<td>Adenomatous, except stomach: fundic gland polyps</td>
<td>Stomach: 23%-100% Duodenum: 50%-90% Jejunum: 50% Ileum: 20% Colon: 100%</td>
<td>33 years</td>
<td>100% (39 years) AFAP, 80% (50 years)</td>
<td>Desmoid tumors, epidermoid cysts, fibromas, osteomas, congenital pigment of the retinal epithelium, dental abnormalities</td>
</tr>
<tr>
<td>HNPCC</td>
<td>Mismatch repair genes</td>
<td>Adenomatous</td>
<td>Colon: 2- to 3-fold sporadic rate</td>
<td>Approximately 44 years</td>
<td>80% (44 years)</td>
<td>None</td>
</tr>
<tr>
<td>Juvenile polyposis syndrome</td>
<td>MADH4, BMPR1A</td>
<td>Juvenile</td>
<td>Stomach and small bowel: may occur Colon: usually</td>
<td>18.5 years</td>
<td>9% to 68% (34 years)</td>
<td>Macrocephaly, hypertelorism, 20% congenital abnormalities in sporadic type</td>
</tr>
<tr>
<td>Cowden’s syndrome</td>
<td>PTEN</td>
<td>Juvenile, lipomas, inflammatory, ganglio-neuromas</td>
<td>Esophagus: 66% (glycogenic acanthosis) Stomach: 75% Duodenum: 37% Colon: 66%</td>
<td>Not known</td>
<td>Approximately 9%</td>
<td>Facial tricholemmomas, oral papillomas, multinodular goiter, fibrocystic breast disease</td>
</tr>
</tbody>
</table>

NOTE. Table summarizes information gained from references.2,7,8,13,106

Genetics of FAP. Ninety percent of families with FAP and its variants arise from mutations of the adenomatous polyposis coli (APC) gene.2,19 The APC gene is a tumor-suppressor gene, and the APC protein is part of the Wnt-signaling pathway, involved in cell growth control.20,28 There also appear to be other functions of the APC gene, including regulation of cell migration up the colonic crypt.20,28 Mutational inactivation of the APC gene causes constitutive activation of the Wnt-signaling pathway and uncontrolled cell growth. Affected persons inherit 1 mutated allele, and a polyp occurs after the second allele is somatically inactivated so that gene function is lost. Approximately 95% of APC mutations that lead to FAP are either nonsense (28%) or truncating frameshift (67%).20,29 The remainder include large deletions or rearrangements and sometimes whole allele deletions.30

APC gene inactivation is the first step in carcinogenesis in FAP but also in more than 50% of common colon adenomas and 80% of adenocarcinomas.28,31 Multiple additional genes are mutated as the clone of cells with loss of APC function progresses toward cancer. This pathogenesis pathway is called the “chromosomal instability” (CIN) pathway because frequent loss of chromosomal material, called loss of heterozygosity (LOH), is observed and believed to be the mechanism of many of the subsequent mutations and gene inactivations.31,32 A distinct pathway is believed to be associated with MYH mutations.33 This pathway involves loss of base excision repair (BER), which may explain the unique molecular features of tumors occurring in patients with MYH-associated polyosis (MAP).

There is some correlation between the location of mutation in the APC gene and the clinical phenotype of FAP.2,34 AFAP arises from APC mutations at the extreme proximal or distal end of the gene or in certain areas of exon 9.2,23 Extreme polyposis, on the other hand, is observed when mutations are in the midportion of exon 15 (the central portion of the gene). Desmoid tumors and osteomas are more common with mutations in the distal portion of the gene.2

Genetic testing for FAP. Genetic testing should be considered in a person who exhibits typical FAP to identify the disease-causing mutation in the family. However, testing also should be considered in persons with as few as 10 adenomas because of the possibility of AFAP or MAP.1,2,24,35 Genetic testing is done by sequencing the APC gene to identify disease-causing mutations, which may be proceeded by CSGE, SSCP, or DGGE to indicate and localize the mutation. Because the large
### Table 7. Gene Frequency, Genetic Testing, Colon Cancer Risk, and Colon Screening Guidelines for Inherited GI Colon Cancer Syndromes

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Gene (frequency mutation is found in index case)</th>
<th>Age to consider genetic testing when the disease-causing mutation in the family is known</th>
<th>Indication for genetic testing when mutation not already known</th>
<th>Colon cancer risk (average age of colon cancer diagnosis)</th>
<th>Colon cancer screening</th>
<th>Upper gastrointestinal screening</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAP</td>
<td>APC (80% to 90%)</td>
<td>10 to 12 years, late teens for attenuated FAP</td>
<td>20 or more adenomatous polyps</td>
<td>100% (39 years) attenuated FAP, 80% (50 yr)</td>
<td>Annual sigmoidoscopy beginning at age 10 to 12 years, colonoscopy beginning in late teens if attenuated FAP is the condition in the family</td>
<td>EGD+ Every 1 to 3 years, depending on severity of duodenal polyposis, start at age 20 to 25 years</td>
</tr>
<tr>
<td>HNPCC</td>
<td>Mismatch repair genes (50% to 70%)</td>
<td>25 years, sometimes younger</td>
<td>Amsterdam I or II or revised Bethesda guidelines (see text and Tables 8 and 9)</td>
<td>80% (44 yr)</td>
<td>Every 2-year colonoscopy starting at age 25 years</td>
<td>Possibly EGD+ every 1 to 2 years, especially if gastric cancer has occurred in other family members</td>
</tr>
<tr>
<td>Peutz–Jeghers syndrome</td>
<td>STK11 (LKB1) (30% to 70%)</td>
<td>When symptoms occur or late teens if symptoms have not occurred</td>
<td>Any Peutz–Jeghers polyp or typical pigmentation</td>
<td>39% (46 yr)</td>
<td>Colonoscopy every 3 years starting with symptoms or late teens if symptoms have not occurred</td>
<td>EGD+ and small bowel examination every 2 years, beginning at age 10 years</td>
</tr>
<tr>
<td>Juvenile polyposis syndrome</td>
<td>MADH4, BMPR1A (40% to 60%)</td>
<td>When symptoms occur or early teens if symptoms have not occurred</td>
<td>3 or more juvenile polyps or extra-colonic juvenile polyps</td>
<td>9% to 68% (34 yr)</td>
<td>Colonoscopy every 3 years starting with symptoms or early teens if symptoms have not occurred</td>
<td>EGD+ and small bowel examination every 2 years, beginning at age 15 years</td>
</tr>
<tr>
<td>Cowden’s syndrome</td>
<td>PTEN (80% to 90%)</td>
<td>By age 25 years, mostly for breast cancer screening</td>
<td>Typical findings or typical colonic polyps</td>
<td>Approximately 9%</td>
<td>None given</td>
<td>EGD+ and small bowel examination every 2 years, beginning at age 15 years</td>
</tr>
</tbody>
</table>

**NOTE.** Table summarizes information gained from references.2–7,8,13,106

*Esophagogastroduodenoscopy

*See Kadmon et al.147

*See Schreibman et al.8

The majority of APC gene disease-causing mutations is either nonsense or frameshift, approximately 95% of mutations can be found by sequencing.4 Southern blotting identifies additional large deletion or rearrangement mutations.36,37 Additionally, reduced protein expression may give rise to disease,18 but causative mutations can be very difficult to find because they may be in regulatory areas (see above). PTT may also be done to indicate the presence of a disease-causing mutation.39 Finally, linkage testing to the APC gene can be done if other approaches are not successful. Available DNA linkage markers are sufficiently accurate that linkage testing can be used in >95% of FAP families with >98% accuracy.6

Of typical FAP or suspected AFAP patients in whom APC mutations cannot be found, 10% to 20% will be found to have mutations of the MYH gene.15,35,40,41 Although rare, this situation is noteworthy because it results in a somewhat less severe colonic polyposis and occurs as an autosomal recessive condition. Genetic testing for MYH mutations is first done by mutation specific testing because 80% of affected persons have 1 of 2 specific MYH mutations: Y165C and G382D.35,40,42 If one of these mutations is present, then sequencing is done to identify an inactivating mutation of the opposite allele because both alleles must be mutated to inactivate the gene and cause disease. If neither of the 2 common mutations is found, but an MYH etiology is still suspected, then primary sequencing can be done to detect other less common mutations.

Patients with multiple but many fewer adenomas than would lead to the suspicion of FAP are also observed to arise from MYH mutations.15,35,41,43 Multiple colonic adenomas caused by MYH mutations will also be discussed in the section on common familial colon cancer.

**Hereditary Nonpolyposis Colorectal Cancer**

The disease and clinical management. HNPCC does not have a distinctive clinical phenotype but is suspected when there is a strong family history of colon cancer and/or colon cancer diagnosis under the age of 50 years.3,4,14,44 It is clinically defined by the Amsterdam criteria, given in Table 8.45,46 Of all colon cancers, 2.6%
will be found in a setting meeting Amsterdam I criteria and 5.5% meeting Amsterdam II criteria. However, only 1% to 3% of colon cancers arise from identified mutations of one of the mismatch repair genes and thus would be said to have genetically defined HNPCC. A larger fraction of young-age-onset colon cancers arises as a part of HNPCC.

The importance of HNPCC is that it accounts for a substantial fraction of colon cancer cases and exhibits an approximate 80% lifetime risk of this malignancy in affected patients. The average age of colon cancer diagnosis is 44 years. There is also an approximate 40% risk of endometrial cancer and 10% risk of ovarian cancer. There is also an approximate 40% lifetime risk of this malignancy in affected patients. The average age of colon cancer diagnosis is 44 years. There is also an approximate 40% risk of endometrial cancer and 10% risk of ovarian cancer. There is also an approximate 40% lifetime risk of this malignancy in affected patients. The average age of colon cancer diagnosis is 44 years. There is also an approximate 40% risk of endometrial cancer and 10% risk of ovarian cancer.

Subtotal colectomy is usually recommended when a colon cancer is diagnosed in a person with HNPCC because of the frequent occurrence of both synchronous and metachronous large bowel cancers. A current alternative recommendation, however, is that patients can be managed after partial colectomy with colonoscopy every 1 to 2 years.

**Genetics of HNPCC**

HNPCC arises from mutations of one of the mismatch repair genes, given in Table 1. Mismatch repair is a mechanism for repair of DNA errors that occur during replication. Replication errors are also called “mismatches” and usually consist of the addition or deletion of 1 or several nucleotides. If one of the mismatch repair genes is inactivated by mutation, mismatch repair does not function properly, and replication errors persist rather than being repaired.

The replication errors or mismatches that are not repaired in HNPCC are most frequently observed in short segments of DNA in which mono-, di-, or trinucleotide repeats of nucleotides occur. These segments are called microsatellites. Tumors that arise when there is loss of mismatch repair gene function exhibit frequent errors in these microsatellites and are thus said to exhibit microsatellite instability (MSI) or be MSI positive. By convention, 5 specific microsatellite loci are evaluated for mutation, or instability, when determining whether a tumor exhibits MSI. The tumor is said to be MSI stable if none of the loci are mutated and to be MSI low if only 1 of the 5 contains mutations. If 2 or more of the microsatellites are mutated, the tumor is called MSI high. Various centers evaluate more than 5 loci, sometimes up to 10. A tumor is considered MSI high or unstable if more than 40% of the loci show instability.

Replication errors or mismatches may affect many genes, but they are often different genes than those found mutated in the CIN pathway, ie, the pathway of mutation accumulation that is observed in tumors when the APC gene is the gene first inactivated. The carcinomaogenesis pathway related to mismatch repair dysfunction is appropriately called the MSI pathway.

**Genetic Testing for HNPCC**

**Amsterdam criteria.** When the Amsterdam I criteria are met, a disease-causing mutation of 1 of the
mismatch repair genes can be found by DNA sequencing in approximately 50% to 70% of cases. Larger DNA deletions and rearrangements appear to be a fairly common cause of HNPCC. Southern blotting done following failure to find a mutation by sequencing finds such disease-causing mutations in an additional 10% to 20% of persons. \(^{(68-70)}\) Over 90% of the mutations found occur in 1 of the 2 mismatch repair genes, \(MLH1\) or \(MSH2\). \(^{(3,6,71)}\) Thus, these are the genes first evaluated when genetic testing is ordered. Another approximate 5% to 10% will have mutations found in the \(MSH6\) gene, and only a rare family will arise from mutations of the \(PMS2\) gene. \(^{(36,72,73)}\)

Genetic testing is possibly even more useful in HNPCC compared with the other colon cancer syndromes because of the lack of a specific clinical phenotype. However, this lack of phenotype has also made the task of determining which patients and families should have genetic testing particularly difficult. Several different but complementary approaches have thus been developed to determine which patients with colon cancer or a family history of colon cancer should be offered genetic testing to confirm a diagnosis of HNPCC. \(^{(48,74-81)}\)

The Amsterdam criteria are used initially to define which families should have genetic testing. \(^{(81)}\) The sensitivity and specificity of the Amsterdam I criteria to detect HNPCC (ie, families with disease-causing mismatch repair gene mutations) are 54% to 91%, and 62% to 84%, respectively. \(^{(82)}\) Sensitivity and specificity figures for the Amsterdam II criteria are 78% and 48% to 68%, respectively. \(^{(82)}\) As seen from the sensitivity figures, if only the Amsterdam criteria are used to determine who should have genetic testing, a substantial fraction of families with HNPCC will be excluded from consideration. Other approaches to determine those who should undergo genetic testing have thus been developed.

**MSI testing of tumor tissue.** One approach in determining who should have genetic testing is based on testing colon cancer tissue for the presence of MSI. \(^{(48,77,80)}\) Paraffin-embedded tissue is completely adequate for this testing. If tumor MSI testing is positive, defined as MSI high, then genetic testing on peripheral blood DNA is next performed to examine for mismatch repair gene mutations. The rational for this approach is that almost all colon cancers in the setting HNPCC exhibit MSI. However, approximately 15% of sporadic colon cancers also exhibit MSI. \(^{(83)}\) MSI in sporadic cancers arises from acquired or somatic dysregulation of the mismatch repair genes. The usual cause of this somatic inactivation of mismatch repair activity in sporadic MSI tumors is aberrant methylation of the \(MLH1\) promoter. Because 15% of sporadic colon cancers exhibit MSI, the presence of tumor MSI does not diagnose HNPCC but makes the diagnosis considerably more likely. However, if the tumor shows no evidence of MSI, germ-line mutation in \(MLH1\) or \(MSH2\) is very unlikely.

MSI is not as often present in uterine and other HNPCC cancers and therefore MSI testing is not usually done in them. The reason for the differences in the frequency of MSI between colon cancers and extracolonic cancers in HNPCC may reflect the fact that microsatellite loci that have been included in the panel of microsatellite repeats used for MSI colon tumor testing were chosen on the basis of their accuracy to detect loss of MMR activity in colon cancers. It is suspected that different loci undergo mutations in the extracolonic tumors. MSI testing in colon adenomatous polyps, however, is often useful but in a subset of polyps. The sensitivity for MSI to be present in adenomatous polyps is almost equivalent to that of colon cancers if the polyp exhibits “advanced” features, including size >1 cm, villous histology, or severe dysplasia. \(^{(84)}\) Smaller polyps without advanced histology often do not exhibit MSI, even if they are removed from persons with HNPCC.

**Bethesda guidelines and MSI testing.** If all colon cancers were tested for MSI, and those patients who had positive tests went on to genetic testing, approximately 6 persons or families would need to have genetic testing for every 1 that would be found to have a germ-line mismatch repair mutation. \(^{(48,85)}\) Because of expense and other issues related to genetic testing, such a broad approach is still considered too expensive and inefficient. Thus, when MSI is being considered as a genetic testing screen, it is recommended that family history and other criteria also be used to limit the number of colon cancer cases to which MSI testing should be applied. \(^{(48,77,80)}\)

A consensus panel has examined various family history, personal history, and tumor histology issues to establish a list of factors that would serve to most effectively and efficiently determine which colon cancer patients should have MSI tumor testing. Genetic testing would then follow if the colon cancer exhibited MSI. These guidelines are called the Bethesda guidelines. \(^{(86,87)}\) They are given in Table 9. If a patient meets any one of the guidelines, MSI testing should be done on the colon cancer tissue. If MSI is absent, HNPCC arising from germ-line mutations of \(MLH1\) or \(MSH2\) is effectively ruled out. If MSI is present, the patient should then undergo genetic testing, looking for inherited mutations of one of the mismatch repair genes. A meta-analysis found that the pooled sensitivity for the Bethesda guidelines to detect mutation positive HNPCC was 89% with a specificity of 53%. \(^{(82)}\) In one study of
Table 9. The Revised Bethesda Guidelines for Testing Colorectal Cancer Tumors for Microsatellite Instability (MSI)

| Tumors from an individual should be tested for MSI in the following situations: |
| Colorectal cancer diagnosed at an age less than 50 years. |
| Presence of synchronous or metachronous colorectal cancer, or other HNPPC-associated tumors (endometrial, stomach, ovarian, pancreas, ureter and renal pelvis, biliary tract, and brain tumors—usually glioblastoma, sebaceous gland adenomas and keratoacanthomas, and carcinoma of the small bowel), regardless of age. |
| Colorectal cancer that exhibits MSI-H histology (presence of tumor-infiltrating lymphocytes, Crohn's-like lymphocytic reaction, mucinous/signet-ring differentiation, or medullary growth pattern) diagnosed in a patient who is less than 60 years of age. |
| Colorectal cancer diagnosed in 1 or more first-degree relatives with an HNPPC-related tumor, with 1 of the cancers being diagnosed under age 50 years. |
| Colorectal cancer diagnosed in 2 or more first- or second-degree relatives with HNPPC-related tumors, regardless of age. |

NOTE. Adapted from Umar et al.87

MSI, microsatellite instability.

sequential colon cancers, 19% of the patients met Bethesda guidelines.11 However, when using conventional scoring criteria, MSI is not always present with HNPPC colon cancers that arise in persons with MSH6 germ-line mutations.58,88–90 This apparent discrepancy can occur because MSH6 germ-line mutations appear to result in MSI mainly at a specific type of microsatellite repeat, mononucleotide repeats, which only account for a subset of the microsatellite repeats used in the MSI panel.

An alternative approach the physician may choose is to go directly to genetic testing when one of the Bethesda guidelines is met, especially if tumor tissue is not available. This direct approach was shown in one study to have a sensitivity of 94% for identification of mutation-positive HNPPC families, with a specificity of 49%.79 A clinical advantage of this approach is that it is a single-stage approach, which can save weeks to sometimes months. Additionally, if tumor tissue is not available for MSI testing, it may be the only option.

**Immunohistochemistry testing of tumor tissue.** Immunohistochemistry (IHC) staining of colon cancer tissue represents a similar approach to MSI.91,93 It is also most efficiently applied when one of the Bethesda guidelines is met. With IHC, specific staining is done on tumor tissue for each of the 4 mismatch repair proteins. Failure of staining of a particular protein indicates lack of expression of that protein and therefore dysfunction of the related mismatch repair gene. Genetic testing, using peripheral blood DNA, should then be done on the indicated gene to find the mutation. Colon cancer tissue is examined first for the expression of MLH1 and MSH2, then MSH6, and, finally, PMS2 if each of the first 3 show normal staining, which infers normal expression of the protein.94 However, if MLH1 is absent, then PMS2 expression will also be absent, so loss of PMS2 as the result of a germ-line mutation can only be assessed if MLH1 is present.

Advantages of IHC testing compared with MSI are that it is inexpensive, can be done in most pathology laboratories, and can direct sequencing toward a particular gene. Its sensitivity and specificity in one study for indicating MLH1 and MSH2 disease-causing mutations were 92.3% and 100%, respectively.95 Its predictive value for MSI-low or MSI-stable tumors in the same study was 96.7% and for MSI-high tumors was 100%. Disadvantages are that it is not as sensitive or specific as MSI. Approximately 10% of IHC tests will be falsely negative, ie, protein stain is present even though the related gene is mutationally inactivated.92,95,96 There are also instances of false-positive results, although less common.96 Finally, laboratories have shown variability in their ability to perform properly the IHC testing.97

In view of the issues with both IHC and MSI, many groups (including the authors') consider these tests somewhat complementary and apply both MSI and IHC when tumor tissue is being examined as part of the genetic testing algorithm.96,98 Applying both overcomes most of the sensitivity and specificity issues of IHC and also finds patients with MSH6 mutations where MSI may be stable.99

Another use of both MSI and IHC testing is application in high-risk patients who have initially undergone genetic testing, but no mutation was found, or a mutation of uncertain significance was found.99 In this instance, if either or both MSI and IHC indicate an underlying mismatch repair dysfunction, the likelihood of HNPPC is much higher. Clinical management should take this finding into account.

**Selection of the index case for genetic testing.** In HNPPC, the person selected as the index case for genetic testing in the family should be the person most likely to carry a disease gene. This would be the youngest person with colon cancer whenever possible. When this is not possible, an older person with colon cancer or with uterine or one of the other HNPPC cancers can be selected. However, the older a cancer patient is, the more likely that the cancer could be a sporadic cancer. Failure to find a mutation in an older cancer patient thus even has less meaning than in a younger patient, but, once a mutation is detected in the index case, mutation-specific testing can be applied to all other family members who desire it.
Testing approach for the patient with a suggestive family history, but no cancer. Possibly the most common logistical issue in approaching genetic testing for HNPCC, especially for the gastroenterologist and primary care physician, is that the person presenting with concern does not have cancer or even colonic polyps. However, the concern comes from that person’s strong family history of colon and other cancers. The optimal solution in this situation is still to identify the youngest person in the family with colon cancer for initial genetic testing. Family members need to be contacted by the patient, not by the physician, in view of current privacy and ethical considerations.

However, if the person who is optimal for testing is either not available or is not willing to consider testing, then other approaches need to be considered. MSI testing on tumor tissue from a living or deceased person who has had colon cancer is usually considered next. The unaffected patient with strong family history can also be considered for primary genetic testing, but the likelihood of that person having a mutation decreases geometrically as relatedness to the cancer patient becomes more distant. An immediate or first-degree relative of a person with known HNPCC, for example, has only a 50% chance of carrying a mutated gene and second-degree relative, a 25% chance. When this is combined with a 50% to 70% chance of initially finding a mutation in an index case, the chances of finding a mutation diminishes further. Thus, the advisability of initial genetic testing in an unaffected person must be put into proper perspective. In our experience, after genetic counseling, unaffected family members need to be contacted by the patient, not by the physician, in view of current privacy and ethical considerations.

However, if the person who is optimal for testing is either not available or is not willing to consider testing, then other approaches need to be considered. MSI testing on tumor tissue from a living or deceased person who has had colon cancer is usually considered next. The unaffected patient with strong family history can also be considered for primary genetic testing, but the likelihood of that person having a mutation decreases geometrically as relatedness to the cancer patient becomes more distant. An immediate or first-degree relative of a person with known HNPCC, for example, has only a 50% chance of carrying a mutated gene and second-degree relative, a 25% chance. When this is combined with a 50% to 70% chance of initially finding a mutation in an index case, the chances of finding a mutation diminishes further. Thus, the advisability of initial genetic testing in an unaffected person must be put into proper perspective. In our experience, after genetic counseling, unaffected first-degree relatives in this situation still often desire testing, whereas second-degree relatives do not.

Summary of testing approach in HNPCC. A summary of the genetic testing approaches to HNPCC is shown in Figure 1. It should also be remembered that genetic testing on the basis of Bethesda guidelines alone, thus bypassing MSI/IHC tumor analysis, also remains a relatively efficient approach, especially if colon cancer tissue is not available for testing. If the approaches shown are followed, an estimated 10% to 15% of persons with colon cancer will undergo genetic screening (MSI/IHC) or direct testing to find the approximately 1% to 3% who actually have genetically defined HNPCC. In families in which no mutation is found, the physician must decide on clinical grounds whether HNPCC is still sufficiently likely to justify HNPCC recommended screening or whether patients can be managed as having increased familial risk for colon cancer but not HNPCC. The results of MSI and IHC testing can assist in this regard. Screening recommendations are given below for familial high-risk colon cancer.

Figure 1. Approach to genetic testing for HNPCC. If the family meets Amsterdam I or II criteria, one should proceed directly to genetic testing to find inherited mutations in one of the mismatch repair genes. If there is a family history of colon cancer, but Amsterdam criteria are not met, then Bethesda guidelines are evaluated. If any are met, microsatellite instability testing (MSI) and/or immunohistochemistry (IHC) testing is done on the colon cancer tissue. If the tumor is MSI positive or mutation of one of the mismatch repair genes is indicated by failure of IHC staining, then genetic testing should be undertaken. Proceeding directly to genetic testing if any of the Bethesda guidelines are met is another approach that can be used, especially if tumor tissue is not available. Modified from Umar et al, with permission.

Peutz–Jeghers Syndrome

The disease. The prevalence of Peutz–Jeghers syndrome is approximately 1 in 200,000 persons. It is autosomal dominantly inherited and characterized by histologically typical polyps, mostly in the small bowel, but also throughout the GI tract, and typical perioral pigmentation. The importance of this condition arises from the benign complications of the polyps, including bleeding, intussusception, and abdominal pain, which usually begin in the second decade of life, and from the substantial malignancy risk that has only recently been appreciated. The lifetime colon cancer risk approaches 40%, whereas breast cancer risk may be over 50%. There is also a substantial risk of pancreatic, gastric, and small intestinal cancer as well as risks of non-GI malignancies, including cancers of the ovary, lung, cervix, uterus, and testicles. Clinical features are summarized in Table 6, and genetic testing, colon cancer screening, and upper gastrointestinal screening guidelines are given in Table 7. Neoplastic changes actually occur in the gastrointestinal hamartomatous polyps, giving rise to the cancer risk. Aggressive screening for extracolonic malignancies has also been recommended for those with Peutz–Jeghers syndrome.

Genetics and genetic testing. Approximately 60% of persons with clinically defined Peutz–Jeghers syndrome will be found to have disease-causing mutations of the STK11 gene, a tumor suppressor gene that is involved in transduction of intracellular growth signals. As many as 25% of cases are not familial, and
thus could represent new mutations or low-penetrance variants. Genetic testing is done by sequencing, often with presequencing methods as described above, although RNA methods have also been suggested. Other genes may also be involved in the genetic etiology of this condition in view of the relatively low rate of finding mutations in the STK11 gene.

Genetic testing is of questionable clinical importance in this condition because the clinical features are usually definitive at a young age. Nonetheless, sporadic Peutz–Jeghers polyps occur, and their presence may prompt the need for genetic testing to determine whether germ-line mutations are present.

**Juvenile Polyposis Syndrome**

**The disease.** Juvenile polyposis syndrome occurs in approximately 1 in every 100,000 persons. As in the other syndromes, it is inherited in an autosomal dominant fashion. It is clinically suspected when 3 to 10 juvenile polyps are found in the colon, or juvenile polyps are found outside the colon. The polyps are found most often in the colon but may occur throughout the GI tract. Malignancy arises from adenomatous change in the juvenile polyps. Patients with juvenile polyposis syndrome exhibit benign complications from the polyps early in life but have a colon cancer risk approaching 60% over a lifetime. Gastric, small intestinal, and pancreatic cancers also occur. Phenotypic features are given in Table 6 and genetic testing and colon and upper gastrointestinal screening guidelines in Table 7. Screening guidelines have been suggested both for the colon and for upper gastrointestinal polyps and malignancies.

**Genetics and genetic testing.** Approximately 15% of juvenile polyposis syndrome patients are found to have mutations of the MADH4 gene, whereas 25% have mutations of the BMPRIA gene and possibly 5% from the PTEN gene. There is some question as to whether those with PTEN mutations actually have Cowden’s syndrome. Both MADH4 and BMPRIA are involved in intracellular signaling processes and mediate signaling from the bone morphogenetic proteins (BMPs), which are members of the TGF-β superfamily. The normal function of BMPRIA is to decrease crypt formation and polyp growth through the Wnt-signaling pathway. It is suspected that other genes, possibly of the TGF-β superfamily, may also give rise to this condition. Approximately 25% of newly diagnosed juvenile polyposis syndrome patients appear to represent de novo or new mutations.

Because sporadic juvenile polyps occur in 2% of children, and the inherited condition does not exhibit a specific phenotype, genetic testing may be extremely helpful in making a diagnosis. Because symptoms often occur early in life, genetic testing is recommended in the first decade when the condition is known to be present in the family. It is also recommended for any person with 3 or more juvenile polyps, even when family history is not suggestive. Genetic testing is done by DNA sequencing of the relevant genes to search for disease-causing mutations.

**Cowden’s Syndrome**

**The disease.** Cowden’s syndrome is an autosomal dominantly inherited disease characterized by the presence of facial trichelemmomas, oral papillomas, multinodular goiter, fibrocystic breast disease, esophageal glycogenic acanthosis, and frequent intestinal hamartomatous polyposis. The colonic polyps are most often histologically juvenile but may also be lipomas, inflammatory polyps, or ganglioneuromas. Cowden’s syndrome occurs in approximately 1 in every 200,000 persons, exhibits a near 50% risk of breast cancer, and a 10% risk of thyroid cancer. It seldom causes gastrointestinal symptoms, despite the frequent presence of polyps. Some surveys have shown an increased risk of colon cancer, whereas others have not. It is likely that Cowden’s syndrome is substantially under diagnosed because the cutaneous manifestations are often subtle and gastrointestinal symptoms unusual. See Tables 6 and 7 for clinical features and genetic testing and colon and upper gastrointestinal screening guidelines, respectively. Breast cancer screening may be the most important clinical screening in Cowden’s syndrome, followed by thyroid cancer screening.

Bannayan–Ruvalcaba–Riley syndrome has many of the features of Cowden’s syndrome, with the addition of frequently slowed psychomotor development and pigmentedary spotting of the penis. The two conditions are now considered to be part of a spectrum arising from mutations of the PTEN gene.

**Genetics and genetic testing.** Patients meeting the clinical criteria of the disease are found to have identifiable disease-causing mutations in the PTEN gene in as many as 80% of cases. The PTEN gene is a tumor suppressor gene involved in cell growth control. The frequency of de novo mutations is unknown. Genetic testing is recommended when facial trichelemmomas, which are distinctive, are observed or a cluster of findings are suggestive of this condition. Genetic testing is done by DNA sequencing.
Common Familial Colon Cancer

Familial Risk and Inheritance

Only a small fraction of colon cancer cases arise in the setting of the highly penetrant autosomal dominantly inherited colon cancer syndromes. However, colon cancer is one of the most familial of all common malignancies. A family history of colon cancer occurs far more frequently than one would expect by chance, and having relatives with colon cancer increases one’s risk of developing this malignancy (Table 10). It has now been demonstrated that the familial clustering of colon cancer cases arises primarily from inherited factors and that up to one third of colon cancers have inheritance as part of the pathogenesis.

Gene Discovery Efforts and Candidate Genes

The genes that are associated with the more common, but less penetrant, category of familial colon cancer are only now being identified. There are likely multiple genes, and genetic-environmental interactions appear to play a significant role in many of the cases with inherited predisposition. A number of candidate genes are being considered, however, and gene discovery efforts are proceeding. The hope is that genetic testing will also soon be available to identify moderate inherited risk because this group is potentially much larger than the group represented by the syndromes and yet will also require more aggressive colon cancer screening. Modern genetic tools will continue to facilitate this gene discovery effort for susceptibility genes with a modest effect.

One example of a moderate genetic predisposition to colon cancer is AFAP. Mutations at the extreme proximal or distal end of the APC gene, as well as mutations in a particular region of exon 9, result in the condition of AFAP. The cancer risk in AFAP is still high, 50% to 80%, but not as high as in typical FAP. Other specific mutations of the APC gene have also been described that give rise to a modest increased risk of colon cancer, estimated at 1.5- to 2.0-fold, including the I1307K mutation and the E1317Q mutation. It has also been suggested that other genes involved in the Wnt-signaling pathway, the pathway in which APC is a part, and even mild mutations of the MMR genes may give rise to polyp and cancer predisposition.

Biallelic MYH gene mutations are found in 40% of persons with 15 or more adenomatous polyps and are therefore clinically similar to AFAP. A small fraction of these patients have more than 100 adenomas and therefore may be confused with typical FAP before genetic testing is done. Patients with multiple colonic adenomas resulting from biallelic MYH mutations are now referred to as MAP. Possibly as many as 1% of colon cancers arise from MAP and 3% of early age colon cancers. The exact cancer risk associated with biallelic MAP patients is not known. It is also not known

Table 10. Familial Risk of Colon Cancer

<table>
<thead>
<tr>
<th>Familial setting</th>
<th>Approximate lifetime risk of colon cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>General population risk in the United States</td>
<td>5%</td>
</tr>
<tr>
<td>One first-degree relative with colon cancer</td>
<td>2- to 3-fold increased</td>
</tr>
<tr>
<td>Two first-degree relatives with colon cancer</td>
<td>3- to 4-fold increased</td>
</tr>
<tr>
<td>First-degree relative with colon cancer diagnosed at ≤50 years</td>
<td>3- to 4-fold increased</td>
</tr>
<tr>
<td>One second- or third-degree relative with colon cancer</td>
<td>Approximately 1.5-fold increased</td>
</tr>
<tr>
<td>Two second-degree relatives with colon cancer</td>
<td>Approximately 2- to 3-fold increased</td>
</tr>
<tr>
<td>One first-degree relative with an adenomatous polyp</td>
<td>Approximately 2-fold increased</td>
</tr>
</tbody>
</table>

NOTE. Modified from Burt, with permission.

*First-degree relatives include parents, siblings, and children.
Second-degree relatives include grandparents, aunts, and uncles.
Third-degree relatives include great-grandparents and cousins.

Figure 2. Colon cancer and inheritance. This Figure illustrates the relative fractions of colon cancer cases that arise from one of the inherited syndromes, from the common familial category, labeled as “other inherited,” or from cases that are said to be sporadic. Reprinted with permission from Burt.
whether monoallelic MYH mutations are associated with adenoma or colon cancer risk.

Some candidate genes have been found to associate with colon cancer risk only when considered together with exposure to certain environmental factors. These include certain polymorphisms of folate reductase (interacting with folate, vitamin B12, alcohol), hydrolases (smoking, cooked meat), NAT2 (smoking), and others. An apparently inherited genetic phenomenon, loss of imprinting of the IGF2 gene, is commonly found in persons with colon cancer, in persons with colonic adenomas, and even in persons with only a family history of colon cancer. The TGF-βR-I(6A) allele also has been associated with a moderate increased risk of colon cancer. Genetic testing involving these and other genes may soon become part of the clinical armamentarium for assessing colon cancer risk and determining optimal colon cancer screening.

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Received December 22, 2004. Accepted February 24, 2005. Address requests for reprints to: Randall Burt, MD, Huntsman Cancer Institute at the University of Utah, 2000 Circle of Hope, Salt Lake City, Utah 84112. e mail: randall.burt@hci.utah.edu; fax: (801) 581-3389. Supported by National Cancer Institute grants R01-CA40641 and P01-CA73992 and by the Huntsman Cancer Foundation.