

The Genetics of Colorectal Cancer

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Colon cancer is a common disease that can be sporadic, familial, or inherited. Recent advances have contributed to the understanding of the molecular basis of these various patterns of colon cancer. Germline genetic mutations are the basis of inherited colon cancer syndromes; an accumulation of somatic mutations in a cell is the basis of sporadic colon cancer; and, in Ashkenazi Jewish persons, a mutation that was previously thought to be a polymorphism may cause familial colon cancer. Mutations of three different classes of genes have been described in colon cancer etiology: oncogenes, suppressor genes, and mismatch repair genes. Knowledge of many of the specific mutations responsible for colon carcinogenesis allows an understanding of the phenotypic manifestations observed and forms the basis of genetic test-

ing for inherited disease. Although genetic testing is possible and available, it is only an adjunct to the clinical management of persons at risk for colon cancer and patients with colon cancer. As a result of advances in the understanding of the molecular causes of colon cancer and the availability of colon cancer screening methods such as colonoscopy, it should be possible to prevent the vast majority of colon cancer in our society. Practicing clinicians should recognize the patterns of clinical colon cancer, understand its causes, and be able to use genetic testing and endoscopic screening for prevention.

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Colon cancer is a common disease in both men and women. Because 5% of persons (1 in 20 persons) will develop colorectal cancer, this disease is an important public health issue. Colon cancer is usually observed in one of three specific patterns: sporadic, inherited, or familial. Sporadic disease, with no familial or inherited predisposition, accounts for approximately 70% of colorectal cancer in the population. Sporadic colon cancer is common in persons older than 50 years of age, probably as a result of dietary and environmental factors as well as normal aging.

Fewer than 10% of patients have an inherited predisposition to colon cancer. The inherited syndromes include those in which colonic polyps are a major manifestation of disease and those in which they are not. The polyposis syndromes are subdivided into familial adenomatous polyposis and the hamartomatous polyposis syndromes. The nonpolyposis predominant syndromes include hereditary nonpolyposis colorectal cancer (HNPCC) (Lynch syndrome I) and the cancer family syndrome (Lynch syndrome II). Although uncommon, these syndromes provide insight into the biology of all types of colorectal cancer.

The third and least understood pattern of colon cancer development is known as familial colon cancer. In affected families, colon cancer develops too frequently to be considered sporadic colon cancer but not in a pattern consistent with an inherited syndrome. Up to 25% of all cases of colon cancer may fall into this category.

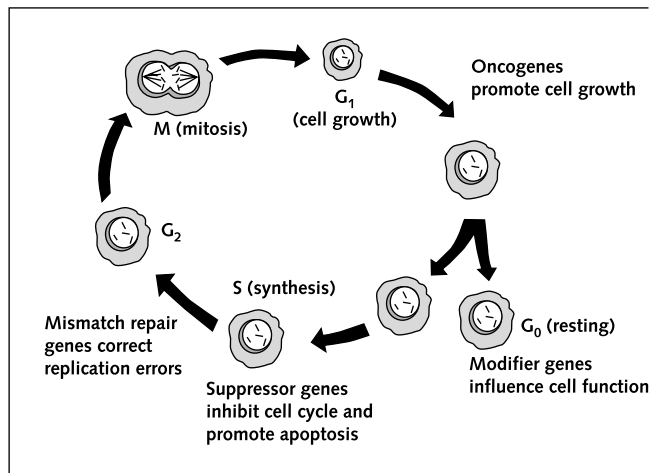
BASIC GENETICS

The complement of DNA in the genetic code is a cell's guide to differentiation and proliferation. All cells of an organism have DNA that is virtually identical to the DNA found in the zygote. A mutation at or before this point of development is therefore termed a germline mutation. A mutation of the ova or sperm is transmitted from the parent as an inherited defect and is responsible for hereditary cancer. When a mutation occurs spontaneously in the

sperm, ova, or zygote, the affected person's parents will not manifest a cancer syndrome. Successive generations, however, can inherit this de novo mutation because the abnormality can be passed on to progeny.

More commonly, a spontaneous mutation occurs in a cell during the growth and development of a particular tissue or organ. This somatic mutation results in clonal proliferation of the cell containing the mutated genetic material. Sporadic colorectal cancer results from the accumulation of multiple somatic mutations in a cell (1). Genes commonly mutated in human cancer belong to one of three different classes: oncogenes, tumor suppressor genes, and mismatch repair genes (2, 3). Oncogenes are normal genes responsible for the stimulation of controlled cellular proliferation (4, 5) (Figure 1). When these genes are mutated, they result in uncontrolled proliferation and, ultimately, cancer. Tumor suppressor genes were first described in Knudson's study of the epidemiology of childhood retinoblastoma (6, 7). Knudson used the term *antioncogene* because the gene was thought to produce cancer in a recessive fashion at the cellular level, meaning that one normal gene was adequate to control cellular growth. Subsequently termed *suppressor genes*, these are normal genes whose function is lost when both copies (alleles) of the gene are inactivated (Figure 1). When a tumor suppressor gene is inherited as a germline mutation, only the mutation of the remaining normal allele is required for the gene's loss of function (Figure 2, top) (5, 6). When both copies of the gene are normal, two mutation events, or hits, are required before the gene loses function (Figure 2, bottom). This two-hit hypothesis explains why inherited disease usually manifests at an earlier age than sporadic disease, as well as the concept of suppressor genes producing cancer in a recessive fashion at the cellular level (7). Enzymes that monitor newly formed DNA and correct replication errors are called DNA mismatch repair (MMR) systems (Figure 1) (3). Defective MMR genes are associated with the so-called mutator phenotype. Cells with MMR

Figure 1. The normal function of the different classes of cancer-causing genes according to cell-cycle stage.



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mutations in both gene copies accumulate DNA errors throughout the genome, affecting growth regulatory genes, such as the transforming growth factor receptor (*TGF- β II*) gene (8). These genes are mutated in HNPCC.

Recently, subtle genetic changes that do not affect protein structure have been recognized as a possible cause of familial colon cancer. These subtle changes, termed *polymorphisms* if they occur frequently in the population and do not affect protein structure, are slight variations of nucleotide base sequences in genes. They are more common in populations than the hereditary cancer gene mutations but do not usually result in clinical disease. One polymorphism, now thought to be a mutation because it ultimately results in an abnormal protein structure, is found on codon 1307 of the *APC* gene and is known as the I1307K *APC* mutation (9). This mutation is found in 6% of all Ashkenazi Jewish persons and in 28% of Ashkenazi Jewish persons with both a personal and family history of colon cancer (Figure 3) (5, 9).

MOLECULAR GENETICS

Fearon and Vogelstein (1) have described the molecular basis for sporadic colon cancer as a multistep model of carcinogenesis. This model describes an accumulation of genetic events, each conferring a selective growth advantage to an affected colon cell. These changes ultimately result in uninhibited cell growth, proliferation, and clonal tumor development. The cumulative effect of these somatic mutations is the cause of sporadic colon cancer.

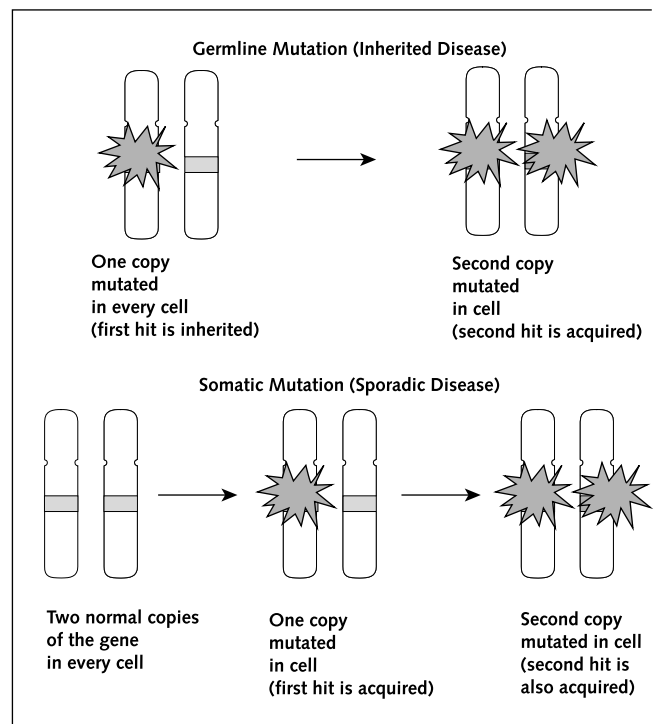
Four main conclusions are drawn from the proposed model of sporadic colon cancer pathogenesis: 1) Colorectal cancer results from the mutational activation of oncogenes and the inactivation of tumor suppressor genes; 2) somatic mutations in at least four or five genes of a cell are required

for malignant transformation; 3) the accumulation of multiple genetic mutations rather than the sequence of mutations determines the biological behavior of the tumor, although *APC* mutations usually occur early in the process and mutations of the *p53* suppressor gene usually occur late in the process; and 4) features of the tumorigenic process of colon cancer are applicable to other solid tumors, such as breast and pancreatic cancer (10).

The most commonly inherited colon cancer syndromes are familial adenomatous polyposis and HNPCC. Each of these syndromes is the result of a specific germline mutation. In familial adenomatous polyposis, the germline mutation is always the *APC* gene, a tumor suppressor gene. In HNPCC, one of the MMR genes is mutated, most commonly *hMLH1* or *hMSH2*. Several of the hamartomatous polyp syndromes have recently been associated with germline mutations. One example is the Peutz-Jeghers syndrome, which results from an abnormality of the *STK11* tumor suppressor gene (11).

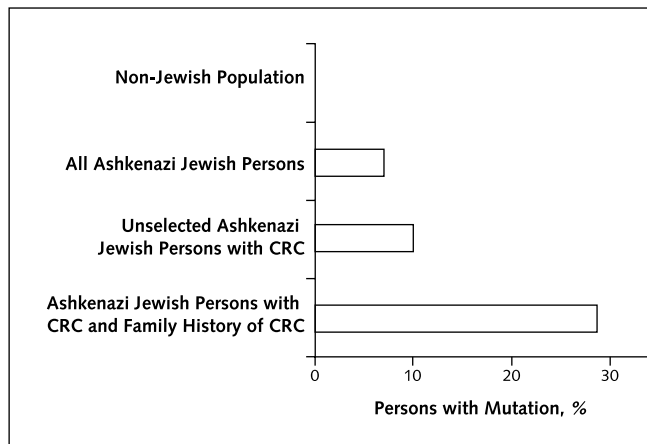
Familial colon cancer in Ashkenazi Jewish persons is probably the result of an I1307K *APC* germline mutation, although the relative risk for tumor is much lower in a person with this mutation than in a person with one of the germline mutations noted previously (Figure 3). Unlike most germline mutations, which cause protein structure abnormalities, the I1307K *APC* germline mutation causes a predisposition to sporadic mutations at distant sites of the gene (which then cause protein structure abnormalities) at a later stage of development.

Figure 2. Loss of suppressor-gene function.



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Figure 3. Familial colorectal cancer (CRC) and APC gene mutation I1307K in Ashkenazi Jewish persons.



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SPECIFIC MUTATIONS

Oncogenes

The oncogene *ras* on chromosome 12 codes for a binding protein that acts as a one-way switch for the transmission of extracellular growth signals to the nucleus and regulates cellular signal transduction. Post-translational modification of the *ras* protein by farnesylation is necessary for activation. Mutations of *ras* are detected in up to 50% of cases of sporadic colorectal cancer and in large polyps. Activation of *ras* leads to constitutive activity of the protein, which results in a continuous growth stimulus that can be the basis of carcinogenesis. Recognition of *ras* mutations may be helpful in screening and early diagnosis of colorectal cancer (12). The usefulness of a sensitive assay for the detection of *ras* mutations in the stool of patients with curable colorectal tumors has been studied (13). Therapeutic potential also exists because clinical trials of farnesyl transferase inhibitors, which specifically inhibit *ras*-mediated signal transduction, have been initiated in patients with colorectal cancer exhibiting *ras* mutations. The *src* oncogene was first identified in Rous sarcoma virus. It encodes for a transforming protein that directly modifies the cytoskeleton. Disruption of the cytoskeleton may be an early event in the process of malignant transformation and carcinogenesis (14, 15). Other oncogenes implicated in sporadic colon cancer include *c-myc* and *c-erbB2* (Table 1) (16, 17).

Tumor Suppressor Genes

The normal function of the *APC* gene is thought to be the modulation of the β -catenin protein, which regulates cell signal transduction and growth (18). The *APC* gene inhibits β -catenin, which controls cellular proliferation. Several cell-signaling pathways converge with the one for *APC* and may result in the same final carcinogenic event. As a result, *APC* mutations are important in early cell

transformation, and *APC* is known as the “gatekeeper” gene (19). When *APC* is a germline mutation, it results in familial adenomatous polyposis; as a somatic mutation, *APC* is an early event in the development of sporadic colon cancer; and as an I1307K mutation, *APC* contributes to the development of familial colon cancer in Ashkenazi Jewish persons (Table 1).

Of the known tumor suppressor genes, *p53* is the most commonly mutated in human cancer (20). Normal *p53* acts by causing G₁ cell-cycle arrest to facilitate DNA repair during replication or to induce apoptosis (programmed cell death). This gene is therefore referred to as the “guardian of the genome.” Up to 75% of sporadic colorectal tumors exhibit *p53* inactivation. Identification of *p53* mutations in colorectal cancer has prognostic significance. Persons with tumors that have a *p53* mutation have worse outcome and shorter survival than persons whose tumors do not have a *p53* mutation (21).

In 1989, a candidate gene known as the “deleted in colorectal carcinoma” (*DCC*) gene was identified (22). Seventy percent of colorectal carcinomas have been shown to have *DCC* mutations on the long arm of chromosome 18. The *DCC* gene encodes a protein that is thought to have a role in cell–cell or cell–matrix interactions. Mutations of the *DCC* gene are also found in 50% of cases of advanced adenoma. The 5-year survival rate seems to be equivalent in patients with stage II colorectal cancer that does not express *DCC* protein product and in patients with more advanced stage III tumors (23). This information may be useful in selecting a subgroup of patients with stage II colorectal cancer who might benefit from adjuvant chemotherapy (Table 1). Recent evidence suggests that *DPC4* (deleted in pancreatic cancer), adjacent to *DCC*, may be

Table 1. Gene Mutations That Cause Colon Cancer*

Mutation Type	Genes Involved	Type of Disease Caused
Germline	<i>APC</i>	Familial adenomatous polyposis
Somatic	<i>MMR</i>	HNPCC (Lynch syndrome)
	Oncogenes	Sporadic disease
	<i>myc</i>	
	<i>ras</i>	
	<i>src</i>	
	<i>erbB2</i>	
	Tumor suppressor genes	
	<i>p53</i>	
	<i>DCC</i>	
	<i>APC</i>	
	<i>MMR</i> genes	
	<i>hMSH2</i>	
	<i>hMLH1</i>	
<i>hPMS1</i>		
<i>hPMS2</i>		
<i>hMSH6</i>		
<i>hMSH3</i>		
Genetic polymorphism	<i>APC</i>	Familial colon cancer in Ashkenazi Jewish persons

* *DCC* = deleted in colorectal carcinoma; HNPCC = hereditary nonpolyposis colorectal cancer; *MMR* = mismatch repair.

the actual suppressor gene that is lost by mutation of chromosome 18 (24). The actual gene that is mutated in colon cancer has not been determined with certainty because it is difficult to find mutations in *DCC*, which is a very large gene.

Mismatch Repair Genes

All of the MMR genes (*hMLH1*, *hMSH2*, *hMSH3*, *hPMS1*, *hPMS2*, and *hMSH6*) are involved in correcting errors of DNA replication (3, 25–27). Mutations of these genes result in abnormal sequences of parts of the DNA known as microsatellites. Microsatellites consist of small sequences of nucleotide bases that are repeated dozens to hundreds of times. The resulting abnormalities of these microsatellites are referred to as microsatellite instability (MSI). Microsatellite instability is frequently seen in colon cancer tissue from patients with HNPCC, which is caused by a germline mutation of one of the MMR genes. As a result, many microsatellite loci have been studied to determine which are most frequently affected in HNPCC. Most laboratories use a panel of several microsatellite loci, although the most sensitive indicator of MSI is the BAT26 locus. Because panels of loci are used, MSI is referred to as absent, low (MSI-L), or high (MSI-H), depending on the number of loci in the panel that demonstrate instability (28). Persons with germline mutations of an MMR gene typically have MSI-H, although the *hMSH6* mutation can be associated with MSI-L (29). Although 10% to 15% of cases of sporadic colon cancer can exhibit MSI, it is usually MSI-L (28, 30).

Modifier Genes

In addition to the genes described, several other genes seem to be important in colon carcinogenesis, although their exact roles and mechanisms of action have not been fully determined (Figure 1). Cyclooxygenase (COX)-2 is one of two COXs, the other being COX-1. Although COX-1 is a constitutive component of cells, COX-2 is induced in colon cancer cells. The COX-2 enzyme probably has a role in programmed cell death. This gene is important because related antagonists are readily available and may be useful in prevention and regression of colon polyps and colon cancer. One nonspecific COX inhibitor, sulindac, has been shown to cause polyp regression in patients with familial adenomatous polyposis (31). Studies of the specific COX-2 inhibitors are ongoing.

The peroxisome proliferator-activating receptor gene (*PPAR*) has also been implicated in colon carcinogenesis. The *PPAR* is a family of nuclear receptors that serve as transcription factors. Preliminary studies demonstrate that *PPAR* may be downstream of the *APC* gene and may also be involved in the COX pathway (32, 33). This could partly explain how mutations of *APC* cause cellular carcinogenesis.

Several receptors in the G-protein receptor family have been implicated in colon cancer growth. Among those studied are the receptor for gastrin and the muscarinic cho-

linergic receptor (34–36). Gastrin is a known trophic factor for colonic mucosa and may have a role in tumorigenesis in the setting of hypergastrinemic states or when abnormal receptors are present. Although gastric acid suppression with proton-pump inhibitors, such as omeprazole, can cause elevated serum gastrin levels, no data currently suggest a causative role for these agents in colon carcinogenesis. Recent studies show that bile acids bind to muscarinic cholinergic receptors, specifically the M3 subtype, and that this type of receptor enhances growth of colon cancer cells (37). Epidemiologic studies have demonstrated a role for bile acids in colon carcinogenesis, and these data may provide insight into the mechanism by which bile acids predispose persons to colon cancer (38–44).

INHERITED SYNDROMES

The common inherited colon cancer syndromes account for approximately 5% to 10% of all cases of colon cancer. Inherited colon cancer is usually the result of a single germline mutation. The phenotypic manifestations of the cancer syndrome depend on the specific gene that is mutated.

Familial Adenomatous Polyposis

In familial adenomatous polyposis, a dominantly inherited syndrome, affected persons develop hundreds to thousands of colonic polyps. Although the rate of transition to cancer is low, the vast number of polyps virtually assures colon cancer development at a young age. Most case-patients identified have a family history, but one third of cases arise spontaneously, the result of a de novo *APC* germline mutation. The genetics of familial adenomatous polyposis is relatively simple because only the *APC* gene is implicated in the disease. Germline mutations of the gene are the molecular cause of familial adenomatous polyposis and its variants, such as the Gardner syndrome. Almost all mutations of the *APC* gene cause premature termination or truncation of its protein product. This provides the basis for the most frequently used genetic screening test, the in vitro protein truncation test for *APC*, which has been commercially available since 1994 (45). Colorectal cancer will develop in virtually all affected patients by the sixth decade of life if prophylactic colectomy is not performed.

HNPCC

The criteria for this syndrome were established in 1991 in Amsterdam by the International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer (46). Before the genetic basis for HNPCC was known, the Amsterdam criteria were specifically designed to be exclusive, or strict (Table 2). This was done to ensure that researchers investigating the genetic basis of HNPCC were studying individuals and families with the same syndrome. Once the MMR genes were determined to be the abnormality causing HNPCC, more clinically relevant, inclusive criteria were established. The Amsterdam criteria were

modified, and subsequent criteria were developed as the result of a workshop sponsored in 1996 by the U.S. National Cancer Institute. The latter guidelines are referred to as the Bethesda criteria (Table 2) (28, 47–49).

In HNPCC, affected persons have a very high risk for colorectal cancer but do not develop the hundreds of polyps seen in familial adenomatous polyposis. Although persons with HNPCC have few polyps, these polyps are very likely to transition to cancer. The rate of progression is rapid, probably because the polyps start with MMR gene mutations and transform rapidly when *APC* mutations develop, a phenomenon known as *accelerated tumorigenesis*. Although sporadic colon cancer usually arises in colon polyps after a 5- to 10-year period of growth and transformation, in HNPCC this progression can occur within 1 or 2 years. Accelerated tumorigenesis therefore has clinical management implications because persons with HNPCC need to have colonoscopic evaluation every 1 to 2 years, an interval much more frequent than for persons without HNPCC. With earlier identification of persons with HNPCC, we will probably detect more polyps before acquisition of *APC* mutations and transformation.

Risk for colon cancer is as high as 85% in persons with an MMR gene mutation. Women with an MMR gene mutation have a 40% risk for endometrial cancer. Affected persons have an increased risk for cancer of other organs, including the ovary, hepatobiliary system, genitourinary system, pancreas, and small intestine.

The initial observation leading to the understanding of the cause of HNPCC was that HNPCC-associated cancer exhibited MSI, also known as the replication error phenotype. Germline mutations of each of the six known MMR genes have been identified in HNPCC kindreds. Mutations are most commonly seen in the *hMSH2* gene, found on chromosome 2p, or in the *hMLH1* gene, found on chromosome 3p. Mutations of *hPMS1*, *hPMS2*, *hMSH3*, and *hMSH6* (GTBP) account for few reported cases. Hereditary nonpolyposis colorectal cancer tends to be right-sided, and affected persons seem to have better ultimate survival than persons with sporadic colorectal cancer.

GENETIC COUNSELING AND GENETIC TESTING

Because of major advances in the understanding of the molecular basis of colon cancer, large amounts of information have been made available to clinicians and prospective patients by the mass media. As a result, it is important for practicing clinicians to understand the genetics of colon cancer, as well as the role and limitations of genetic counseling and testing, to improve interaction with patients.

Genetic Counseling

The first step in assessing risk in an individual patient is a detailed family history that allows a determination of whether cancer in affected family members is sporadic, familial, or inherited (50). If family history suggests familial clustering of colon cancer or a pattern consistent with fa-

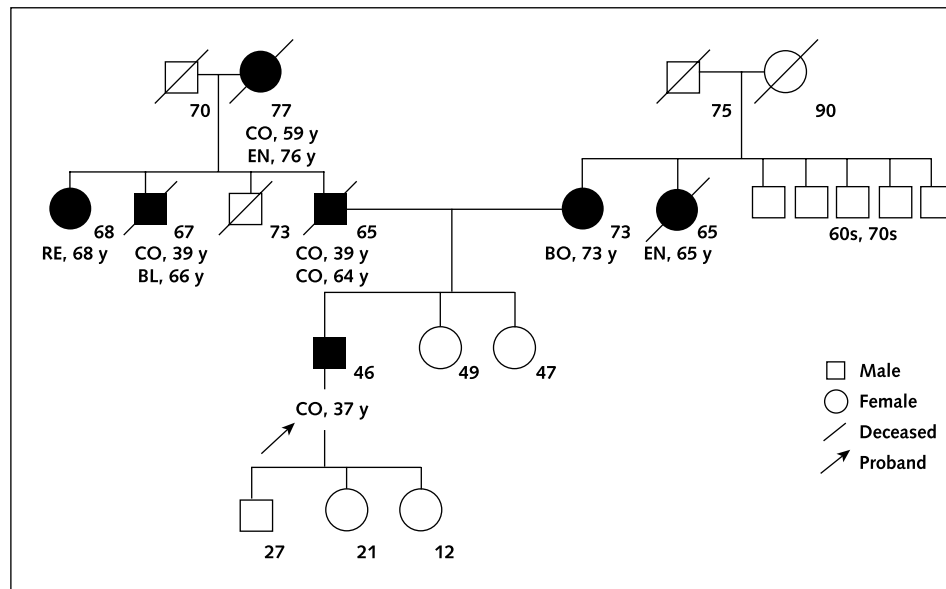
Table 2. Clinical Criteria for Hereditary Nonpolyposis Colorectal Cancer*

Amsterdam criteria
At least three relatives with colon cancer and all of the following:
One affected person is a first-degree relative of the other two affected persons
Two successive generations affected
At least one case of colon cancer diagnosed before age 50 y
FAP excluded
Modified Amsterdam criteria
Same as the Amsterdam criteria, except that cancer must be associated with HNPCC (colon, endometrium, small bowel, ureter, renal pelvis) instead of specifically colon cancer
Bethesda criteria
The Amsterdam criteria or one of the following:
Two cases of HNPCC-associated cancer in one patient, including synchronous or metachronous cancer
Colon cancer and a first-degree relative with HNPCC-associated cancer and/or colonic adenoma (one case of cancer diagnosed before age 45 y and adenoma diagnosed before age 40 y)
Colon or endometrial cancer diagnosed before age 45 y
Right-sided colon cancer that has an undifferentiated pattern (solid-cirriiform) or signet-cell histopathologic characteristics diagnosed before age 45 y
Adenomas diagnosed before age 40 y

* FAP = familial adenomatous polyposis; HNPCC = hereditary nonpolyposis colorectal cancer.

miliar adenomatous polyposis or HNPCC, a genetic counselor who is knowledgeable about genetic testing options should obtain a family pedigree (Figure 4). Since the capability of performing genetic testing is still at an early stage, there remains a fair amount of concern about the ramifications of a positive test result. In a recent survey of members of the National Society of Genetic Counselors Special Interest Group in Cancer, 68% of respondents said that they would not bill their insurance company for their own genetic testing and 26% said that they would be tested under an alias (51). If genetic testing is indicated, it should be preceded by a pretest counseling session detailing the limitations of the test and the potential psychological, ethical, legal, and societal implications for the proband and his or her family members. A disclosure session should be held to reveal the results of testing, followed by a post-test counseling session to ensure that the patient has understood the results and has an opportunity to ask questions. A recent study addressing the use of commercially available *APC* gene testing revealed that despite appropriate patient selection in 80% of cases, genetic counseling was offered in only 20% of cases and one third of physicians misinterpreted the test results (45). In addition, several cases concerning families with HNPCC have resulted in litigation; the outcomes have varied from case to case and have often set legal precedent for a particular state. One such precedent places responsibility on the physician to inform patients' family members if they are at high risk for cancer because of HNPCC, while the precedent in another state places greater emphasis on the patient-physician relationship and does not allow the physician to inform anyone of risk except the patient (52).

Figure 4. Pedigree of a family meeting the Amsterdam criteria for hereditary nonpolyposis colorectal cancer.



Inheritance is from the proband's paternal family. Black shading indicates cancer. BL = bladder cancer; BO = bone cancer; CO = colon cancer; EN = endometrial cancer; RE = rectal cancer.

Genetic Testing

It is possible to perform genetic testing for inherited disorders that result from germline mutations because these abnormalities are found in the DNA of circulating leukocytes. Blood tests can be done to detect germline mutations found in all cells of an individual patient. Basic science laboratories that study the genetics of colorectal cancer can perform genetic testing but do not typically accept samples for clinical purposes. Several commercial enterprises and some pathology laboratories at medical centers offer genetic testing. The sensitivity and clinical applicability of a specific genetic test are limited by the general methodologic techniques used to indirectly detect evidence of a genetic mutation. The only method that directly tests for a gene mutation is gene sequencing, which consequently has high sensitivity. The sensitivity of indirect testing methods depends on whether a person's particular gene mutation causes an abnormality that is detected by the testing method. In addition, the percentage of patients harboring a known gene mutation differs according to the inherited syndrome.

There are three basic mechanisms by which to test for genetic abnormalities. The first, which is used infrequently, is linkage analysis, an indirect method of locating DNA markers that segregate with causative mutations. The advantage of this method is that there is no need to know a specific gene before looking for an inherited syndrome. Linkage analysis requires at least three family members with the disease and results in a mathematical determination of the probability that a disease is being transmitted in a family (Figure 5) (5). The second mechanism takes advantage of the difference in the movement of a mutated protein or DNA on gel electrophoresis. Protein truncation

testing is a transcription–translation method of detecting proteins of abnormal size and depends on whether a mutation results in a shortened protein, which is usually the case for *APC* (Figure 5). In a test tube, a person's DNA is transcribed into RNA and then translated into a protein, which is then run on an electrophoretic blot and compared with normal and abnormal specimens (Figure 5). The DNA itself can also be run on an electrophoretic blot. Single-strand conformational polymorphism and denaturing gradient gel electrophoresis detect the difference in movement of abnormal DNA; sensitivity depends on whether a mutation results in a DNA sequence of abnormal length after enzyme digestion (Figure 5). These methods are the ones most commonly used by commercial laboratories. The third mechanism is direct mutational analysis, which requires sequencing of a specific gene to look for mutations and is typically performed in research laboratories (Figure 5).

Genetic Testing for Familial Adenomatous Polyposis

The *APC* gene is the only germline mutation that leads to familial adenomatous polyposis (Table 1). Mutations of the *APC* gene can be detected by in vitro protein truncation testing. The sensitivity of this test is approximately 65%, and the approximate cost, obtained from information made public by commercial laboratories, is \$750. Once an *APC* mutation is detected, subsequent family members can be tested for approximately \$500.

Genetic Testing for HNPCC

The genetics of HNPCC is more complicated than that of familial adenomatous polyposis because numerous germline mutations of the MMR genes can cause HNPCC

(Table 1) (3, 53). Two of these, *hMSH2* and *hMLH1*, are responsible for disease in approximately 85% of patients with HNPCC. Additional genetic abnormalities are likely to be found that will account for the 15% of patients who do not show abnormalities of the known genes. Because the sensitivity of the clinical test is less than 65%, only approximately 50% of persons in families that meet the Amsterdam criteria will have a positive result on tests for an *hMSH2* or an *hMLH1* mutation. This raises obvious concerns about false-negative results.

In addition to the three described methods of testing for genetic abnormalities, a fourth possibility exists for HNPCC: determining whether there is MSI in tumor tissue. Microsatellite instability occurs only after both copies of the MMR gene are lost, so it is necessary to study tumor tissue. Blood cells will have a functional copy of the MMR gene and will therefore not exhibit MSI. Many laboratories will use tumor tissue to screen and determine whether to proceed with mutational analysis (Figure 5). Another approach would be to use MSI as an initial screening method only in patients whose families do not meet classic Amsterdam criteria but in whom clinical suspicion is high (30). The cost of genetic testing for HNPCC, based on information made public by commercial laboratories, is approximately \$2000. This includes MSI testing as well as tests for *hMLH1* and *hMSH2*.

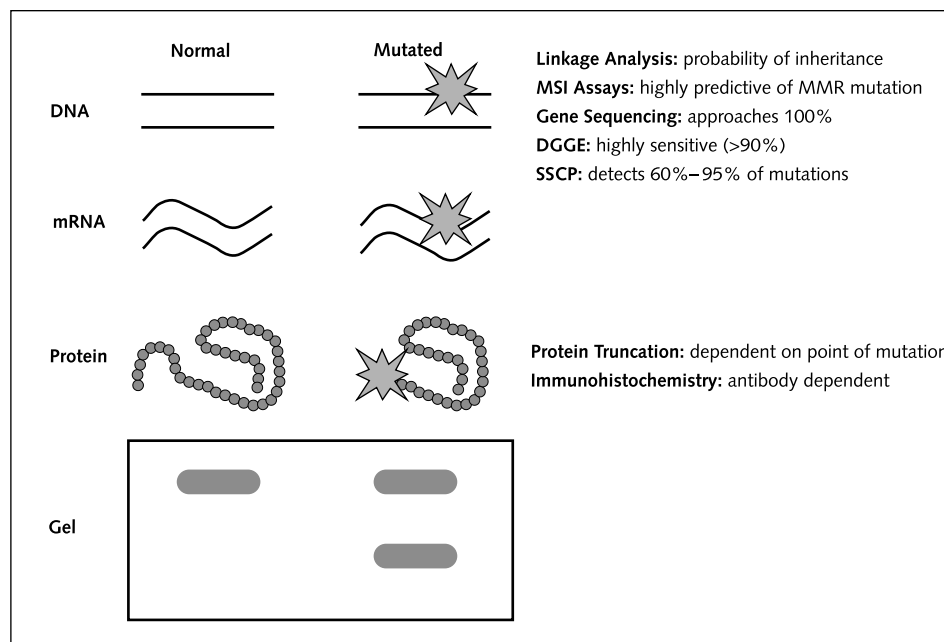
Recently, attempts have been made to produce antibodies that detect *hMLH1* and *hMSH2* proteins. These antibodies serve as the basis of immunohistochemical detection of MMR gene mutations (Figure 5) (54). Immu-

nohistochemistry, which reveals the absence of an MMR protein that presumes a mutation of the gene, has also been proposed as an initial test to determine whether mutational analysis should be performed.

Clinical Application of Genetic Testing

Although genetic testing is slowly becoming more available, its clinical indications are still limited. The benefits of genetic testing include the ability to identify mutation carriers, the ability to identify noncarriers in families with known mutations, and the ability to reduce mortality rates in at-risk persons by vigilant endoscopic surveillance. There are, however, limitations to genetic testing. Current tests do not identify all mutation carriers, noncarriers have a continued risk for colon cancer, and the efficacy for surveillance of other carriers is unknown (5, 55). Genetic testing for an inherited syndrome should be considered when the clinician strongly suspects that a family is exhibiting a syndrome in an autosomal dominant fashion or that an individual is exhibiting specific features of a syndrome, such as hundreds of colon adenomas (familial adenomatous polyposis) or colon cancer before 45 years of age (HNPCC). Genetic testing should be considered as one part of the clinical evaluation of patients who are thought to have inherited colon cancer syndromes (Figure 6). Because genetic testing is expensive, many laboratories expect full payment from patients before performing tests. Many patients will be partially or completely reimbursed by their health insurance carriers, but reimbursement policies vary greatly among carriers and geographic regions.

Figure 5. Laboratory methods for detection of gene mutations, according to the nucleic acid or protein tested.



Although sensitivities for each test vary by laboratory and the specific gene involved, some indication of utility is given for each test. DGGE = denaturing gradient gel electrophoresis; mRNA = messenger RNA; MSI = microsatellite instability; SSCP = single-strand conformational polymorphism. Modified and reprinted with permission from reference 5. Copyright 1998 by the American Society of Clinical Oncology.

The sensitivity of commercially available genetic tests is relatively low, and tests developed for the purpose of detection are not available for all cancer-causing mutations (Figure 5). Therefore, the false-negative rate of genetic tests is high, and only persons who are clinically suspected of having an inherited disease should be tested. If the test results are positive, unaffected first-degree relatives can then be tested with close to 100% sensitivity. If the test results are negative, unaffected first-degree relatives should not be tested because the test will be uninformative.

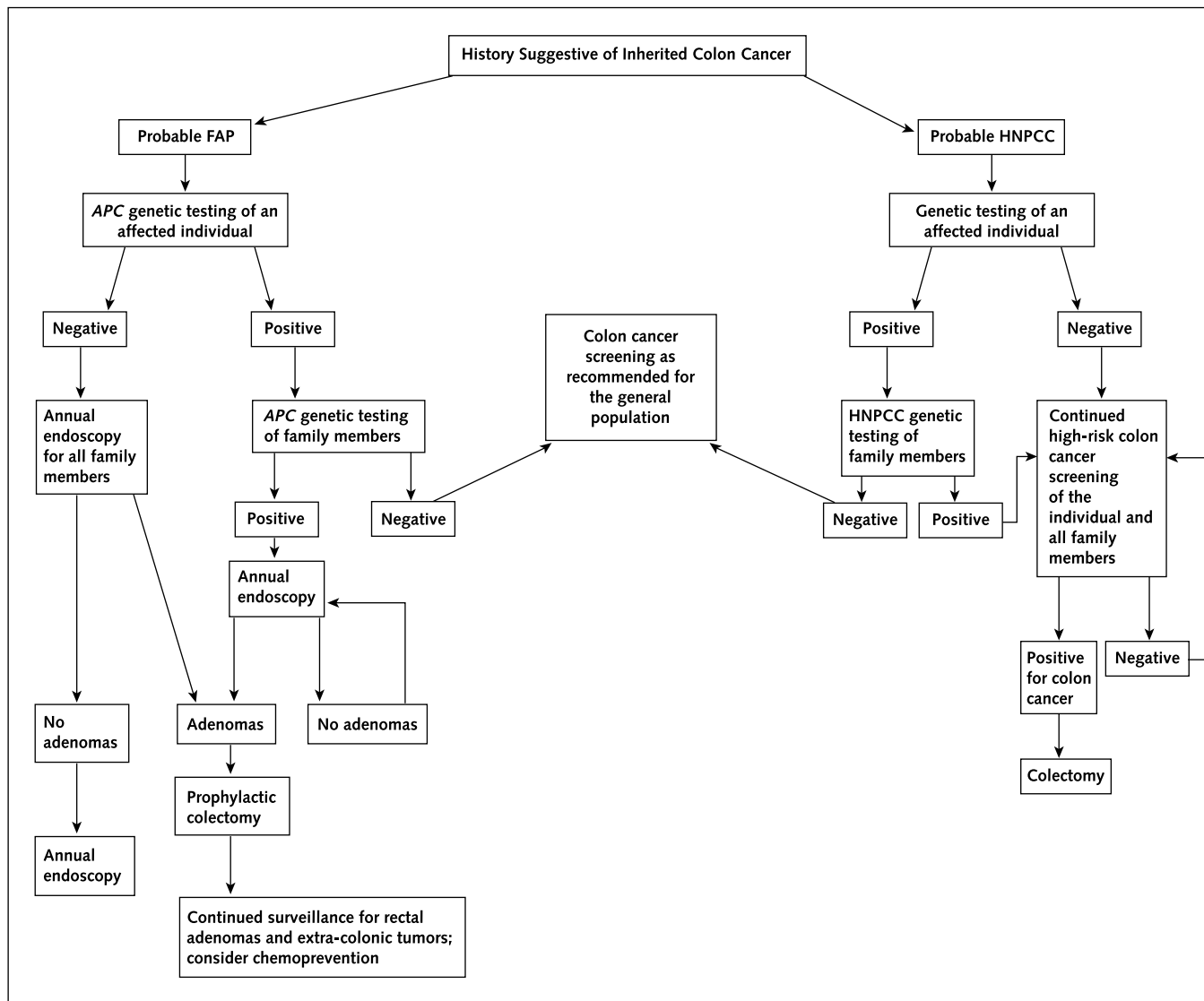
Once it is decided that a clinical genetic test should be performed, the physician must find a laboratory that performs the specific test needed. The list of laboratories that perform the genetic tests discussed in this paper changes rapidly. A genetic counselor can be helpful in identifying laboratories, as can www.genetests.org, a Web site funded by the National Institutes of Health.

Because of the limitations of clinical genetic testing, family history and clinical presentation should drive decisions about colon cancer screening. Several medical organizations have developed and endorsed clinical guidelines for colon cancer screening in different situations. Among these organizations are the American Gastroenterological Association and the American Cancer Society (56). The consensus recommendations, as well as recommendations for additional situations, are given in Table 3 (56–58).

CONCLUSION

Rapid advances are being made in the understanding of the epidemiology and molecular genetics of colon cancer. With these advances come opportunities for clinically applicable genetic tests. These tests are not perfect and are still prone to yielding false-positive and false-negative re-

Figure 6. Algorithm for a clinical approach to inherited colon cancer syndromes.



FAP = familial adenomatous polyposis; HNPCC = hereditary nonpolyposis colorectal cancer.

Table 3. Recommendations for Clinical Cancer Screening*

Disease	Risk Factors	Screening Method	Age at Which To Begin Screening, y	Frequency
Colon cancer	Average risk	FOBT	50	Annually
		Sigmoidoscopy	50	Every 5 y
		FOBT and sigmoidoscopy	50	Annually and every 5 y
		DCBE	50	Every 5–10 y
		Colonoscopy	50	Every 10 y
	First-degree relative with colon cancer or adenomatous polyp at age ≥ 60 y	Same as for average risk	40	Same as for average risk
Extracolonic cancer	Two or more first-degree relatives with colon cancer or adenomatous polyps at age < 60 y	Colonoscopy preferred	40, or 10 y younger than earliest age at which a family member received a diagnosis	Every 3–5 y
	FAP	Sigmoidoscopy	10–12	Annually
	HNPCC	Colonoscopy	20–25, or 10 y younger than earliest age at which a family member received a diagnosis	Every 1–2 y
Duodenal cancer	FAP	EGD	20–25	Every 1–3 y
Endometrial and ovarian cancer	HNPCC	Pelvic examination and transvaginal ultrasonography	25–35	Every 1–2 y
Gastric cancer	HNPCC	EGD	30–35	Every 1–2 y

* DCBE = double-contrast barium enema; EGD = esophagogastroduodenoscopy; FAP = familial adenomatous polyposis; FOBT = fecal occult blood test; HNPCC = hereditary nonpolyposis colorectal cancer.

sults. Because of this, the clinical understanding of colon cancer remains the basis for management decisions, with genetic testing having a potentially important supporting role.

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