

AGA Technical Review on Hereditary Colorectal Cancer and Genetic Testing

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Colorectal cancer is a major American health problem, ranking as the second leading cause of cancer death, after lung cancer, in the United States. About 129,400 new cases are diagnosed each year, and 56,600 Americans die annually from this disorder.¹

The etiology of colorectal cancer is heterogeneous, with environment or genetics playing varying key roles in different patients.² About 80% of patients with colorectal cancer seem to have sporadic disease with no evidence of having inherited the disorder. In the remaining 20%, there seems to be a potentially definable genetic component. Evidence for a genetic contribution to colorectal cancer includes increased risk of colorectal malignancy in persons with a family history and familial aggregation of colorectal cancer consistent with autosomal dominant inheritance.^{3,4} In the past decade, germline genetic mutations conferring high lifetime risk of colorectal cancer in carriers have been found, accounting for 5%–6% of all colorectal cancer cases. Other gene mutations, some with lower lifetime risks, are continuing to be characterized.

The translation into clinical practice of genetic discoveries related to hereditary colorectal cancer continues apace, primarily through improved risk assessment by genetic testing. When used appropriately, genetic testing for hereditary forms of colorectal cancer can confirm or reject the diagnosis at the molecular level, justify surveillance of at-risk persons, decrease the cost of surveillance by risk stratification, aid in surgical and chemoprevention management, and help in decisions regarding family and career planning. But when used inappropriately, genetic testing has the potential to misinform affected patients with false-negative results.⁵

This technical review critically analyzes currently available literature and: (1) succinctly reviews the known colorectal cancer genetic syndromes of familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNPCC) and variants, (2) analyzes the current availability and cost of genetic tests, and (3) describes indications for use of genetic tests in the hereditary colorectal cancer syndromes.

Literature Review

General Information

Study identification and selection. A systematic computer-aided search of MEDLINE and *Current Contents* from January 1966 to December 1999 was performed focusing on the major hereditary colorectal cancer syndromes and associated reports of genetic testing. The search identified all literature under the medical subject headings and text words, “familial adenomatous polyposis,” “adenomatous polyposis,” “adenomatous polyposis coli,” “familial polyposis coli,” “hereditary nonpolyposis colorectal syndrome,” “HNPCC,” “Lynch syndrome,” and “gene/genetic testing.” In addition, an extensive manual search was conducted using references from all retrieved reports, review articles, and chapters from gastroenterology textbooks. Data concerning availability and costs of genetic tests were collected by a search of GeneTests, www.genetests.org, and by telephone survey of specific laboratories. Publications and other information were retrieved, and the authors synthesized and assessed the quality of the available data with respect to topicality and currency. Differences among reviewers concerning inclusion were resolved by consensus. Editorials and letters to the editor were excluded from this review.

Review of Inherited Colorectal Cancer Syndromes

FAP

Clinical features. FAP is an autosomal dominant syndrome caused by germline mutation of the *APC* (adenomatous polyposis coli) gene.^{6–8} This disorder is

Abbreviations used in this paper: APC, adenomatous polyposis coli; FAP, familial adenomatous polyposis; HNPCC, hereditary nonpolyposis colorectal cancer; IHC, immunohistochemistry; MMR, mismatch repair; MSI, microsatellite instability; MSS, microsatellite stable; PTT, protein truncation testing; SSCP, single-strand conformation polymorphism testing.

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estimated to occur in 1 of 8300 to 1 of 14,025 live births, affects both sexes equally, and has worldwide distribution.⁹ Characteristically, teenaged patients develop multiple (≥ 100) adenomas diffusely throughout the colon (Figure 1); 50% of FAP patients develop adenomas by 15 years of age and 95% by age 35.^{10–12} Colorectal cancer is inevitable in FAP patients if colectomy is not performed. The average age at diagnosis ranges from 34.5 to 43 years.^{10,13}

In addition to polyposis coli, patients with FAP can develop a variety of benign extracolonic manifestations (Table 1). These include extracolonic polyps (adenomas of the small intestine and stomach, fundic gland retention polyps of the stomach),^{14–17} cutaneous lesions (lipomas, fibromas, sebaceous, and epidermoid cysts),^{18,19} desmoid tumors,²⁰ osteomas,^{21,22} occult radiopaque jaw lesions,²³ dental abnormalities,²⁴ pigmented ocular fundic lesions (congenital hypertrophy of the retinal pigment epithelium),^{23,25,26} and nasopharyngeal angiofibroma.²⁷ Extracolonic malignancies that can develop in FAP patients include hepatoblastoma,²⁸ upper gastrointestinal tract malignancies (duodenum and periampullary area, very rarely jejunum),¹⁶ thyroid gland, biliary

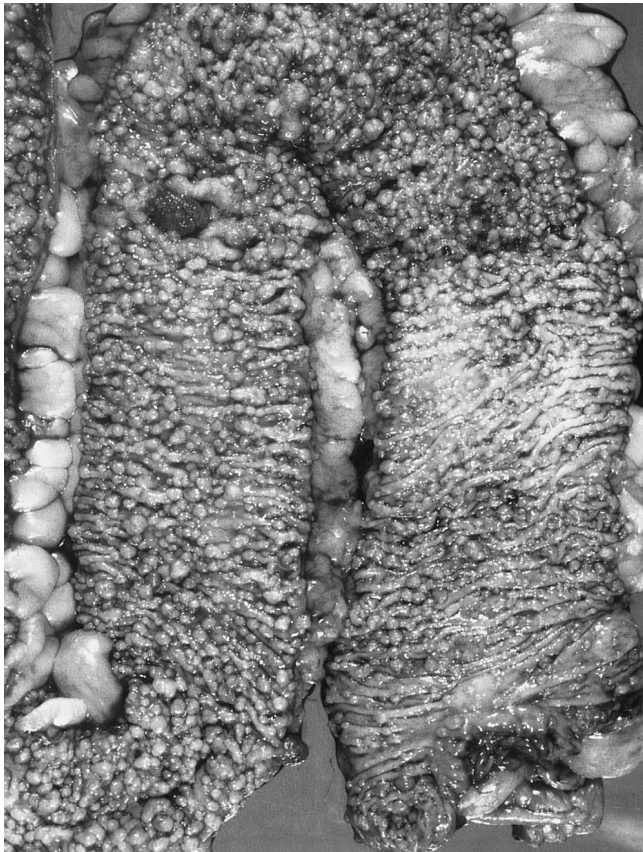


Figure 1. Colectomy specimen demonstrating diffuse polyposis, from a patient with FAP.

tree, pancreas,²⁹ and brain.³⁰ FAP is also known as familial polyposis, and older terminology designated patients with extraintestinal manifestations as having Gardner syndrome.

Genetic defect. Almost all cases of FAP are caused by germline mutations of the *APC* gene on chromosome 5q21.^{6–8,31} The *APC* gene is a tumor suppressor or “gatekeeping” gene with 15 exons that encodes for a 2843 amino acid protein with a molecular weight of 309,000 daltons considered important in cell adhesion, signal transduction, and transcriptional activation.³² Beta-catenin and c-myc have been identified as downstream targets.³² More than 300 different mutations of the *APC* gene causing FAP have been reported.³³ Most are insertions, deletions, and nonsense mutations that lead to frame shifts or premature stop codons, resulting in truncation of the *APC* gene product. The most common mutation, occurring in 10% of FAP patients, is a deletion of AAAAG in codon 1309.

Genotype-phenotype studies reveal that *APC* gene mutations between codons 169–1393 result in classic FAP, more 3' and 5' mutations predispose to attenuated FAP,^{34–36} and mutation I1307K of the *APC* gene increases risk of colorectal cancer among persons of Ashkenazi Jewish ancestry³⁷ (see Attenuated FAP and I1307K *APC* Gene Mutation). Other correlations with mutation include the profuse colorectal polyposis between codons 1250 and 1464,³⁸ predilection for multiplicity of extraintestinal manifestations at codons 1465, 1546, and 2621,³⁹ and occurrence of retinal lesions with mutations restricted to codons 463 to 1444.^{39–42} The tendency for desmoid tumors between codons 1445 and 1578^{41–43} has been reported, although others have not found this association.³⁹ However, considerable intra- and interfamilial phenotypic variability can occur even in persons with identical genotypic mutations.⁴⁴

Management

Screening. At-risk individuals (first-degree relatives of FAP patients; Figure 2) should undergo screening for FAP at ages 10–12.^{12,45} The screening test of choice is genetic testing for mutation of the *APC* gene (see Indications and Strategy for Genetic Testing).^{45,46} If informative genetic testing cannot be done, at-risk members are advised to pursue endoscopic screening with yearly sigmoidoscopy starting at 12 years old, reducing screening frequency with each subsequent decade. After age 50, patients are advised to follow the American Gastroenterology Association guidelines for screening average-risk patients.⁴⁵

Hepatoblastoma occurs in about 1 in 300 persons at risk for FAP under the age of 5 years (1 in 150 *APC* gene

Table 1. Extracolonic Features in FAP and HNPCC

Syndrome	Cancers	Other lesions
FAP	Brain (medulloblastoma) ^a	CHRPE
	Thyroid	Nasopharyngeal angiofibroma
	Duodenal	Osteomas
	Periampullary	Radiopaque jaw lesions
	Pancreas	Supernumerary teeth
	Hepatoblastoma	Lipomas, fibromas, epidermoid cysts
	Biliary tree	Desmoid tumors Gastric adenomas/fundic gland polyps Duodenal, jejunal, ileal adenomas
HNPCC	Brain (glioblastoma) ^b	Cafe au lait spots
	Stomach	Sebaceous gland adenomas, carcinomas ^c
	Small bowel	Keratoacanthomas ^c
	Biliary tract	
	Ureter and renal pelvis	
	Uterus Ovary	

CHRPE, Congenital hypertrophy of the retinal pigment epithelium.

^aCrails syndrome is characterized by medulloblastoma associated with adenomatous polyposis.

^bTurcot's syndrome is characterized by glioblastoma associated with HNPCC.

^cMuir-Torre syndrome is a variant of HNPCC characterized by sebaceous gland tumors and/or keratoacanthomas.

mutation carriers).²⁸ Because of potential cure by early surgery of an otherwise rapidly fatal tumor, screening with serum alpha-fetoprotein levels and imaging of the liver in children of parents affected with FAP from infancy to 5 years of age may be prudent.^{28,47}

Treatment. Colectomy is the recommended therapy to eliminate the development of colorectal cancer in FAP patients. Surgery is usually advised at the time of diagnosis to minimize the risk of malignancy. If patients are in the second decade of life, and adenomas are less than 6 mm and without any villous component, delay to accommodate work and school schedules seems appropriate.^{13,45} Surgical options include subtotal colectomy with ileorectal anastomosis, total proctocolectomy with Brooke ileostomy (or continent ileostomy), and proctocolectomy with mucosal proctectomy and ileoanal pull-through (with pouch formation).⁴⁸⁻⁵⁰ Because colorectal cancer can occur in the rectal segment,^{51,52} most investigators favor the latter 2 procedures.

Postoperative follow-up. Patients with subtotal colectomy require routine endoscopic surveillance of the remaining rectum about every 6 months for recurrent adenomas and/or carcinomas.^{51,52} Although the long-term risk of neoplastic transformation in the ileoanal pouch of patients with restorative proctocolectomies is low, adenomas in the pouch have been reported. In such

cases some experts have recommended that endoscopic biopsy surveillance should be considered.⁵³ Most authorities recommend upper endoscopic surveillance (with biopsy and brushing) of the stomach, duodenum, and periampullary region with front- and/or side-viewing endoscopes every 6 months to 4 years, depending on polyp burden.¹⁶ Annual physical examination of the thyroid is warranted, along with consideration for ultrasonography.²⁹ The use of oral sulindac or celecoxib (a selective COX-2 inhibitor) to prevent or eliminate polyps in the retained rectum has been shown to be effective in the short term.⁵⁴⁻⁵⁶ Although regression of polyps throughout the colon with a selective COX-2 inhibitor has been reported,⁵⁶ no authority has advocated the use of these agents for primary treatment of FAP. Sulindac has also been noted in case reports to regress duodenal adenomas, but a small controlled study showed no benefit.⁵⁵

Attenuated FAP

Genotype-phenotype correlation studies have reported that mutations in the 5' (5' to codon 158) and 3' (3' to codon 1596) ends of the *APC* gene are associated with a less severe phenotype of FAP.^{34,35,57-61} This variant was first termed attenuated adenomatous polyposis coli (AAPC) by Spirio et al. in 1992³⁴ and later renamed

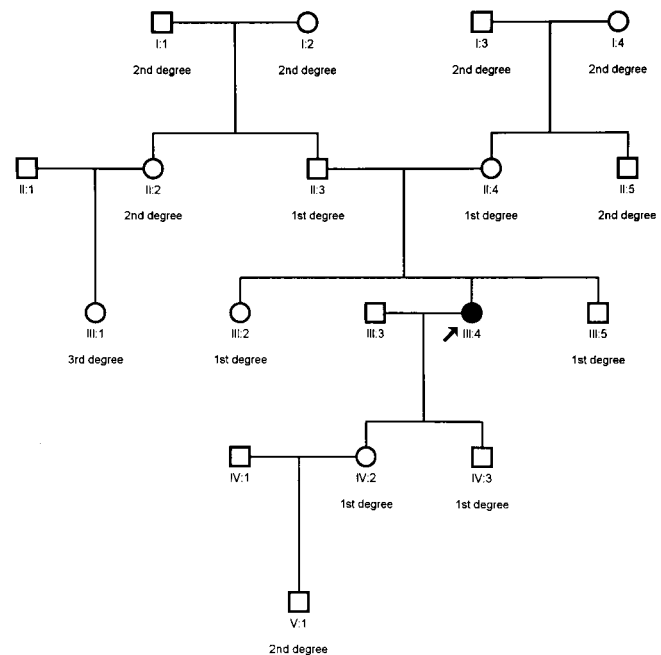


Figure 2. Relationships within a kindred. The arrow indicates the proband, III-4, who has hereditary colorectal cancer. The proband's first degree relatives are her parents (II-3, II-4), her siblings (III-2, III-5), and her children (IV-2, IV-3). The proband's second degree relatives are her grandparents (I-1, I-2, I-3, I-4), paternal aunt and maternal uncle (II-2, II-5), and her grandson (V-1). The proband's third degree relative is her paternal first cousin (III-1).

Table 2. Summary of Commercially Available Genetic Tests for Hereditary Colorectal Cancer

Disorder	Genetic analysis	Method	Sensitivity/accuracy	Cost
FAP	<i>APC</i>	Sequencing	>90%	\$800/unknown mutation; \$200/known mutation
	<i>APC</i>	Linkage	99%	\$245–\$260/person; \$630–\$1750/family
	<i>APC</i>	Protein truncation	70%–80%	\$750–\$1000/unknown mutation; \$500/known mutation
	<i>APC</i>	Protein truncation + CSGE + SSCP + sequencing	80%–90%	\$530/unknown mutation
<i>APC</i> I1307K	<i>APC</i>	ASO	99%	\$150–\$255
	<i>APC</i>	Sequencing	99%	\$250
HNPCC	<i>hMSH2, hMLH1</i>	Sequencing	>90%	\$800–\$3000/unknown mutation; \$200–\$500/known mutation
	<i>hMSH2, hMLH1</i>	CSGE + sequencing	>90%	\$1540/unknown mutation; \$260/known mutation
	<i>hMSH2, hMLH1</i>	DOVAM-S (SSCP)	95%–100%	\$800/unknown mutation; \$250/known mutation
	<i>hMSH2, hMLH1</i>	Protein truncation	50%–65%	\$750/unknown mutation; \$500/known mutation
	MSI testing of tumor	Polyacrylamide gel electrophoresis	N/A	\$300–\$500

NOTE. See www.genetests.org for details concerning specific laboratories.

CSGE, Conformation strand gel electrophoresis; ASO, allele-specific oligonucleotide; DOVAM-S, detection of virtually all mutations-SSCP.

attenuated familial adenomatous polyposis.⁶¹ The clinical characteristics of the attenuated variant include oligopolyposis (fewer than 100 colorectal adenomas) at presentation but marked phenotypic variation within pedigrees, and a delayed onset of colorectal cancer that occurs on average 12 years later than in classic FAP.³⁵ In addition, a plethora of upper tract lesions has been noted in some of these patients.⁶²

I1307K *APC* Gene Mutation

Recently, a missense mutation in the *APC* gene, *APC* I1307K, was discovered as a cause for an undefined proportion of familial colorectal cancer in patients in a specific ethnic group³⁷; this mutation is found in 6% of the general Ashkenazi Jewish population.^{37,63,64} Individuals with this mutation have an increased lifetime risk of colorectal cancer, with an estimated odds ratio of 1.4 to 1.9.^{37,63,64} The mechanism by which the missense mutation predisposes colonic epithelial cells to acquire somatic mutations in the *APC* gene is through the instability conferred by the thymine to adenine transversion, resulting in a polyadenine tract in the gene. There is an increased frequency of somatic mutations in the mutation-bearing allele in colorectal tumors of I1307K carriers.^{37,63,64}

Genetic testing for the *APC* I1307K gene mutation is commercially available for Ashkenazi Jewish individuals with a personal or family history of colorectal cancer (Table 2). Further research is ongoing to delineate the

clinical application of this mutation and other associated phenotypic characteristics. The results of future investigation will determine the advisability of using *APC* I1307K testing in clinical management.

HNPCC

Clinical features. In 1966, Dr. Henry Lynch and colleagues described familial aggregation of colorectal cancer with stomach and endometrial tumors in 2 extended kindreds and termed it Cancer Family Syndrome⁶⁵; later, authors designated this constellation Lynch syndrome. More recently, this condition has been called HNPCC. In HNPCC, unlike FAP, colorectal cancer usually arises from a single colorectal lesion in the absence of polyposis (≥ 100 adenomas).^{66–68}

HNPCC is an autosomal dominant disorder accounting for about 3%–5% (range, 1%–13%)^{69–71} of all colorectal cancer cases caused by mutation of 1 of the DNA mismatch repair genes. Individuals with an HNPCC gene mutation have approximately a 70%–80% lifetime risk of developing colon or rectal cancer.^{72,73} Colorectal cancer occurs at a younger age (average, 44 years old), compared with sporadic cases of people developing cancer in the seventh decade of life.⁶⁸ Colorectal tumors arise primarily (60%–80%) on the right side of the colon (proximal to the splenic flexure); in sporadic colorectal cancer, only 23%–32% are right-sided.⁶⁸ In 45% of affected individuals, multiple synchronous and metachronous colorectal cancers can occur within 10 years of

Table 3. Amsterdam Criteria I and II (International Collaborative Group) for the Diagnosis of HNPCC

Amsterdam criteria I
1. Three or more relatives with histologically verified colorectal cancer, 1 of whom is a first-degree relative of the other 2; FAP should be excluded
2. Colorectal cancer involving at least 2 generations
3. One or more colorectal cancer cases diagnosed before the age of 50
Amsterdam criteria II
1. Three or more relatives with histologically verified HNPCC-associated cancer (colorectal cancer, cancer of the endometrium, small bowel, ureter, or renal pelvis), 1 of whom is a first-degree relative of the other 2; FAP should be excluded
2. Colorectal cancer involving at least 2 generations
3. One or more cancer cases diagnosed before the age of 50

resection.^{66,74} The precursor lesion for HNPCC seems to be a discrete proximal colonic adenoma, which can occasionally appear flat rather than polypoid.^{75,76} These lesions are more often villous, with areas of high-grade dysplasia, than those from the general population and consequently are considered more likely to undergo malignant transformation.⁷⁷ The histopathology of HNPCC colorectal cancers tends to be poorly differentiated, abundant in extracellular mucin, and distinguished by a lymphoid (Crohn's-like pattern or peritumoral lymphocytes) host response to the tumor.^{78,79} HNPCC patients have improved survival from colorectal cancer stage-for-stage compared with those with sporadic tumors.⁸⁰

In addition to colorectal cancer, patients and family members are at jeopardy for a wide variety of extracolonic malignancies (Table 1). The highest risk in HNPCC patients occurs for endometrial and ovarian malignancy (39% and 9% risk by age 70, respectively).⁸¹⁻⁸⁴ Increased relative risk of transitional cell carcinoma of the ureter and renal pelvis and adenocarcinomas of the stomach, small bowel, ovary, and biliary system occurs in these pedigrees (ranging from 3 to 25 times the general population).⁸¹⁻⁸⁴ Also, an excess of pancreas, larynx, breast, brain, and hematopoietic malignancies has been described.^{68,85} Unlike FAP with multiple overt syndromic manifestations, café au lait spots, sebaceous gland tumors, and keratoacanthomas (the latter 2 found in the variant Muir-Torre syndrome) are the only reported phenotypic signs in HNPCC.⁸⁵

In 1990, the International Collaborative Group on Hereditary Nonpolyposis Colorectal Cancer established research criteria (ICG or Amsterdam criteria I) for HNPCC⁸⁶ (Table 3). All of the following are required to diagnose HNPCC: (1) 3 or more relatives with histologically verified colorectal cancer, 1 of whom is a first-degree relative of the other 2 (FAP should be excluded);

(2) colorectal cancer involving at least 2 generations; (3) 1 or more colorectal cancer cases diagnosed before the age of 50. In response to concern that these standards are too stringent, particularly for clinical application and further research, alternative criteria (Amsterdam criteria II) were proposed (Table 3). Amsterdam criteria II include extracolonic tumor types commonly observed in HNPCC, such as cancer of the endometrium, upper gastrointestinal tract, and urinary tract.⁸⁷

Genetic Defect

HNPCC is caused by mutation of 1 of several DNA mismatch repair genes. These genes function to maintain fidelity of DNA during replication by correction of nucleotide base mispairs and small insertions or deletions generated by misincorporations or slippage of DNA-polymerase during DNA replication. The mismatch repair genes include *bMSH2* (human mutS homolog 2) on chromosome 2p16^{88,89}; *bMLH1* (human mutL homolog 1) on chromosome 3p21^{90,91}; *bPMS1* and *bPMS2* (human postmeiotic segregation 1 and 2) on chromosomes 2q31 and 7q11,⁹² respectively; and *bMSH6* (human mutS homolog 6) on chromosome 2p16.^{93,94} Germline mutations of *bMSH2* (frameshift = 60%, or nonsense mutations = 23%) and *bMLH1* (frameshift = 40%, and missense alterations = 31%) account for more than 95% of the mutations found in HNPCC families; they are distributed throughout the 16 and 19 exons of these 2 genes, respectively.⁹⁵

Microsatellite instability (MSI) is a phenomenon found in the colorectal cancer DNA (but not in the adjacent normal colorectal mucosa) of individuals with mismatch repair gene mutations. MSI is characterized by expansion or contraction of short repeated DNA sequences (i.e., microsatellite repeats) caused by insertion or deletion of repeated units. This instability, known as "mutator phenotype," "replication error," or "microsatellite instability," indicates probable defects in the mismatch repair genes caused by somatic changes or can suggest the diagnosis of HNPCC.⁹⁶ MSI has been found in most cases (>90%) of HNPCC that fulfill the Amsterdam criteria⁷¹ and in 15% of sporadic colorectal cancers. The role of MSI analysis has led to the development of the Bethesda criteria, which set forth clinical indications for use of this assay and standardization of tumor analysis (Tables 4 and 5).^{70,97} The Bethesda criteria have been modified in the Medical Position Statement for purposes of genetic testing by changing the guidelines for age of adenocarcinoma diagnosis from less than 45 years old to less than 50 years old. Recently, investigators have found that MSI, as a marker for the mismatch repair pathway of colorectal neoplasia, is asso-

ciated with improved survival, stage-for-stage, in those sporadic patients with MSI-positive tumors.^{80,98} In addition, 1 study reported that adjuvant chemotherapy resulted in greater survival benefits for patients with MSI-positive tumors.⁹⁹

Management

Screening. Recommendations for colorectal cancer screening in HNPCC have been published by several authors^{45,100} on the basis of expert and consensus opinion. In the absence of genetic testing, first-degree relatives (at 50% risk for HNPCC) of affected individuals are recommended to have colonoscopy every 1–2 years, starting between 20 and 30 years of age, and annually after 40 years of age,⁴⁵ or alternatively every 1–2 years, beginning at age 25.¹⁰⁰ Individuals with germline mutations are recommended to start colonoscopy at age 25 or 5 years younger than the youngest age at diagnosis in the family, whichever comes first, and to continue annually.¹⁰⁰ Jarvinen et al. showed that colorectal screening decreases morbidity and mortality from colorectal cancer for the children of patients with HNPCC.¹⁰¹

Annual screening for endometrial cancer is recommended by expert opinion beginning at age 25–35. There is no consensus on the optimal method of screening, but choices include endometrial aspiration or transvaginal ultrasonography.^{68,102} The efficacy of these tools

Table 4. Bethesda Criteria for Testing Colorectal Tumors for MSI

1. Individuals with cancer in families that meet the Amsterdam criteria
2. Individuals with 2 HNPCC-related cancers, including synchronous and metachronous colorectal cancers or associated extracolonic cancers^a
3. Individuals with colorectal cancer and a first-degree relative with colorectal cancer and/or HNPCC-related extracolonic cancer and/or a colorectal adenoma; 1 of the cancers diagnosed at age <45 years,^b and the adenoma diagnosed at age <40 years
4. Individuals with colorectal cancer or endometrial cancer diagnosed at age <45 years^b
5. Individuals with right-sided colorectal cancer with an undifferentiated pattern (solid/cribriform) on histology diagnosed at age <45 years^{b,c}
6. Individuals with signet-ring cell-type colorectal cancer diagnosed at age <45 years^{b,d}
7. Individuals with adenomas diagnosed at age <40 years

^aEndometrial, ovarian, gastric, hepatobiliary, small bowel, or transitional cell carcinoma of the renal pelvis or ureter.

^bGuidelines for age of cancer diagnosis have been adapted to <50 years in the AGA Medical Position Statement (Bethesda criteria modified).

^cSolid/cribriform defined as poorly differentiated or undifferentiated carcinoma composed of irregular, solid sheets of large eosinophilic cells and containing small gland-like spaces.

^dComposed of >50% signet ring cells.

Table 5. International Guidelines for Evaluation of MSI in Colorectal Cancer

Markers	Repeating unit	
BAT25	Mononucleotide	
BAT26	Mononucleotide	
D5S346	Dinucleotide	
D2S123	Dinucleotide	
D17S250	Dinucleotide	
	Number of markers of instability positive	
	5 Loci evaluated	>5 Loci evaluated
MSI-High	≥2	≥30%–40%
MSI-Low	1	<30%–40%
MSS or MSI-Low	0	0

remains uncertain.¹⁰² Some experts have recommended screening for ovarian and genitourinary cancers when these tumors have been observed in the family,^{103,104} although insufficient data exist to evaluate this strategy. Proposed methods of screening for ovarian cancer include transvaginal ultrasonography and serum CA-125, and urine analysis for renal tumors.

Treatment. Subtotal colectomy with ileorectal anastomosis (and postsurgical rectal surveillance) is recommended when colorectal cancer develops in the setting of HNPCC because of the high rate of metachronous colorectal cancer noted in follow-up studies.^{66,102} This operation can be considered for prophylaxis in selected mismatch repair gene mutation carriers with adenomas at time of surveillance and is an option in mutation carriers without mucosal disease.¹⁰²

A consensus panel found insufficient evidence to recommend for or against prophylactic hysterectomy and oophorectomy as a measure for reducing cancer risk¹⁰² and advised that women who are carriers of the HNPCC-associated mutation should be counseled that this is an available option.

Review of Genetic Testing Studies

A detailed literature review of hereditary colorectal cancer genetic testing studies is presented below. Investigators have used a variety of test methodologies to search for mutations in the hereditary colorectal cancer genes. Descriptions of these techniques are presented in the glossary.^{105–107}

FAP

Studies using various methods for identifying APC gene mutations are noted in Table 6. Methodologies for FAP gene testing include linkage analysis, which is informative for 33%–80% of FAP pedigrees^{108,109};

Table 6. Literature Summary of APC Gene Test Results

Population	Method	Mean % positive or informative for linkage (range)	Reference
Affected individuals	PTT with conversion	96	31
	PTT	84 (79–90)	111–113
	PTT with sequencing	90	117
	PTT with DGGE	59 (56–62)	115, 116
	PTT with SSCP	52	114
	Sequencing	67	118
	Linkage	56 (33–80)	108, 109
	SSCP	29	110

DGGE, denaturing gradient gel electrophoresis.

single-strand conformation polymorphism testing (SSCP), which has been reported to detect 29% of mutations in the APC gene¹¹⁰; protein truncation testing (PTT), which ranges from 79% to 90% in sensitivity^{111–113}; and conversion with PTT, which is reported to detect 96% of mutations in the APC gene.³¹ Combinations of mutation screening techniques that combine SSCP, denaturing gradient gel electrophoresis, or sequencing in combination with PTT detect 52% to 90% of mutations.^{114–117} Clinical tests available for FAP are shown on Table 9. Currently, genetic testing using protein truncation is the preferred methodology for FAP gene testing. In contrast to sequencing, which can detect variants of unknown significance, PTT usually detects disease-causing mutations. Also, PTT is generally less costly than sequencing, and turnaround time for results is shorter.

HNPCC

As described above, MSI is a genetic phenomenon found in colorectal tumor DNA. Before 1997, investigators used a variety of markers to show MSI in colorectal cancer specimens. In 1997, the National Cancer Institute Workshop on Hereditary Nonpolyposis Colorectal Cancer Syndrome proposed specific markers for MSI testing (Table 5).^{70,97} Results are classified as MSI-high, MSI-low, or MSS (microsatellite stable), as described in Table 5. MSI is considered to be present in the specimen if the result is MSI-high.

The occurrence of MSI in various patient populations with colorectal cancer varies, as shown in Table 7. The majority of these studies were performed before standardization of MSI testing in 1997. The greatest frequency of MSI was found in the tumors of patients who are clinically affected with HNPCC, by Amsterdam criteria. A significant decrement in MSI positivity occurs in all other populations. Compared with sporadic colorectal cancers, in which MSI is noted in 15% of patients,

individuals with colorectal cancer at young age, suspected HNPCC, and right-sided colorectal cancer have frequencies of MSI positivity ranging from 17% to 58%.

Table 8 summarizes the results of mismatch repair gene testing in several populations at increased risk for colon cancer, using various testing methods. The highest rates of gene mutation were found in families fulfilling the Amsterdam criteria for HNPCC (45%–64%) and in those individuals with colorectal cancer before age 30 and positive MSI in tumor tissue (63%). The range of positivity varied widely, depending on test methodology and number of genes tested. The positivity decreased substantially in those tested from families with suspected HNPCC or those meeting less stringent criteria (0%–47%). A 30% positivity was noted in individuals meeting the Bethesda criteria when analyzing 2 genes (*bMLH1*, *bMSH2*), whereas 10% tested positive when *bMSH6* alone was analyzed. The occurrence of MSI substantially increased the likelihood of detecting a germline mutation, as noted in individuals with colorectal cancer before the age of 50, and before the age of 30 with and without MSI testing.

Wijnen et al. developed a model for assessing the likelihood of germline mutations in HNPCC. Multivariate analysis showed that a younger age at diagnosis of colorectal cancer, fulfillment of the Amsterdam criteria, and the presence of endometrial carcinoma in the kindred were independent predictors of germline mutations of *bMSH2* and *bMLH1*.¹¹⁹

Recent research has found that immunohistochemistry (IHC) for *bMLH1* and *bMSH2* expression is an inexpensive adjunct analysis of colorectal tumors, and when used

Table 7. Literature Summary of MSI Test Results

Population	Mean % MSI positive (range)	References
HNPCC; meets Amsterdam criteria I	81 (44–100)	95, 134–138
HNPCC suspected; positive family history; does not meet Amsterdam criteria I	36 (9–62)	120, 135, 136, 139
Multiple CRC (with or without family history)	35 (26–50)	122, 140, 141
CRC < 30 yr	32	142
CRC < 35 yr	58	143
CRC < 45 yr	21	135
CRC < 50 yr	17	122
Unselected CRC	21 (7–38)	122, 144–151
	Low 25 (22–27)	149, 150, 152
	High 13 (9–16)	
Sporadic right-sided CRC	44 (31–57)	122, 138
Sporadic left-sided CRC	0	122
Sporadic CRC	15 (5–20)	134, 137, 153–156

CRC, Colorectal cancer.

Table 8. Literature Summary of MMR Gene Test Results in Different Risk Populations

Population	Gene(s)	Method	Mean % positive (range)	Reference(s)
HNPPC; meets Amsterdam criteria I	<i>hMLH1, hMSH2, hMSH6</i> <i>hMLH1, hMSH1</i>	Sequencing	63	133
		Sequencing	37 (25–45)	157–160
		PTT	68 (50–86)	161, 162
		SSCP	52 (41–67)	163–166
		DGGE	45 (29–56)	73, 167, 168
HNPPC; meets Amsterdam criteria II	<i>hMLH1, hMSH2</i>	Heteroduplex analysis, RT-PCR, PTT, sequencing	64	132
		Sequencing	18	158
HNPPC suspected ^a	<i>hMLH1, hMSH2</i>	SSCP	0	163
		Sequencing	16	160
Meets Bethesda criteria +	<i>hMLH1, hMSH2</i> <i>hMSH6</i>	PTT ± 2D electrophoresis, SSCP, heteroduplex analysis, sequencing	29–30	162, 169
		SSCP	32 (13–60)	170–172
		DGGE	8	168
		DGGE, PTT, sequencing	47	131
		Heteroduplex analysis, RT-PCR, PTT, sequencing	24	132
CRC < 50 yr, MSI+	<i>hMLH1, hMSH2</i>	Sequencing	30	158
		Sequencing	10	173
CRC < 50 yr	<i>hMLH1, hMSH2</i> <i>hPMS1, hPMS2</i>	SSCP, PTT, heteroduplex analysis	30	169
		Heteroduplex analysis, RT-PCR, PTT, sequencing	0	132
CRC < 40 yr	<i>hMLH1, hMSH2</i>	Sequencing	17	158
		SSCP	2	171
CRC < 30 yr, MSI+	<i>hMLH1, hMSH2</i>	PTT, sequencing	63	174
CRC < 30 yr	<i>hMLH1, hMSH2</i>	PTT, sequencing	28	174
MSI+ sporadic CRC	<i>hMLH1, hMSH2</i>	Sequencing	14	160

DGGE, Denaturing gradient gel electrophoresis; RT-PCR, reverse-transcription polymerase chain reaction; CRC, colorectal cancer.

^aHNPPC suspected is based on family history, but criteria were not specified.

with MSI can help determine strategies for germline testing. Lack of expression of either *bMSH2* or *bMLH1* by IHC in tumors is correlated with MSI in the tumor.^{120–122} The majority of MSI observed in sporadic cases seem to be caused by somatic hypermethylation of the *bMLH1* promoter.^{123,124} It has also been observed that absent *bMSH2* IHC expression is associated with germline *bMSH2* mutation, and a minority of absent *bMLH1* IHC expression is associated with germline *bMLH1* mutation.¹²⁴ IHC for these 2 genes is now transitioning into routine clinical practice.

Indications and Strategy for Genetic Testing

Recommendations for the rational use of genetic tests have been published by several organizations.^{125–127} The American Society of Clinical Oncology (ASCO) recommends that practitioners must ensure that the patient or guardian has given informed

consent.¹²⁵ Experts advise that written informed consent be obtained on test-specific forms. The form should include a general description and purpose of the test, a description of the disorder tested for, the meaning of positive and negative test results, the level of certainty that a positive test result serves as a predictor for the disease, and the actions that persons can take if they receive a positive result. In addition, ASCO advocates that practitioners include pre- and post-test genetic counseling about the possible risks and benefits of early detection of cancer and of prevention modalities with presumed but unproven efficacy for individuals at the highest risk for cancer. Among concerns addressed by counseling in gene-positive patients include anger and denial about the test result, worry about social stigma, greater trepidation of surgery or death, anxiety about interference with work or school, and fear of loss of insurability. Gene-negative patients can develop “survivor guilt”—

Table 9. Indications for Gene Testing

Gene test	Indications
APC gene test	<p>≥100 colorectal adenomas</p> <p>First-degree relatives (10 yr or older) of patients with FAP</p> <p>≥20 cumulative colorectal adenomas (suspected attenuated FAP)</p> <p>First-degree relatives (10 yr or older) of patients with attenuated FAP</p>
MSI testing	<p>Affected individuals in families meeting Amsterdam criteria</p> <p>Affected individuals meeting Bethesda criteria modified</p>
MMR gene testing (<i>hMSH2</i> , <i>hMLH1</i>)	<p>Patients with MSI-high tumor test result</p> <p>Affected individuals in families meeting any of the first 3 criteria of the Bethesda criteria modified or tumor tissue not available</p> <p>First-degree adult relatives of those with known mutation</p>

guilt over escaping an illness that has afflicted other family members. Counseling issues are further explored in other publications.^{125,128,129}

In general, genetic testing should be offered only when: (1) the person has a strong family history of cancer or early age of onset of the disease, (2) the test can be adequately interpreted, and (3) the results will influence the medical management of the patient or family member.¹²⁵

FAP

Genetic testing in FAP is considered the standard of care for management of this disorder.^{100,125}

APC gene testing. Concordant indications for APC gene testing have been developed by several organizations^{45,100} and by expert opinion.^{12,46} These include confirmation of the diagnosis of FAP and presymptomatic diagnosis of individuals 10 years of age or older at risk for FAP (first-degree relatives of those affected) (Table 9, Figure 2).

The strategy for testing of individuals affected and at risk for FAP (presymptomatic testing) is illustrated in Figure 1 of the adjoining Medical Position Statement.^{12,46,100} Evaluation of at-risk persons begins by first testing an affected member of the family to establish a detectable mutation in the pedigree. Several methods for APC gene testing are commercially available, as shown in Table 2. Although sequencing provides a high degree of sensitivity for mutations in the APC gene, the protein truncation test is less labor intensive and has the advantage of selecting for disease-causing mutations. Protein truncation testing is also preferred over linkage analysis because only 1 affected family member is required for

PTT. In individuals affected with FAP, PTT detects 80% of mutations in the APC gene. Future introduction of conversion technology may increase the sensitivity to 96%.^{31,130}

If a mutation is found in an affected family member, then genetic testing in at-risk relatives will provide true positive or negative results. Appropriate screening strategies can then be undertaken, based on the at-risk person's gene test result. If a mutation is not identified, testing at-risk relatives is useless because the gene test will be inconclusive: a negative result could be a false negative because the PTT is not capable of detecting a mutation, even if present. When an affected family member is not available for evaluation, performing the test on at-risk family members can provide only positive or inconclusive results. In this circumstance, a true negative test result for an at-risk individual can only be inferred if another at-risk family member tests positive for a mutation.

Attenuated FAP

Experts agree that APC testing is indicated for confirmational and presymptomatic diagnosis in attenuated FAP, but no clear standard exists concerning the number of colorectal adenomas needed to suspect this diagnosis in the index case. Some experts would test patients with 20 or more cumulative colorectal adenomas.⁵

HNPCC

Medical benefit of genetic testing in HNPCC is presumed but has not been established.¹²⁵

MSI testing. MSI evaluation is indicated for patients who meet the Bethesda criteria modified, as noted in Table 4.⁷⁰

MSI testing of the colorectal tumor (adenocarcinoma or adenoma) serves as a screening test for HNPCC (Table 2). Evaluation for MSI is suggested by consensus panel¹⁰⁰ and expert opinion^{128,129} as the first step in the genetic work-up of pedigrees suspected to be affected by HNPCC (Figure 2 of the adjoining Medical Position Statement). MSI testing is performed on the tumor tissue of individuals putatively affected with HNPCC. A result of MSI-high in tumor DNA provides evidence for the presence of germline mutation in a mismatch repair gene and prompts patient testing for mutation of the *bMSH2* and *bMLH1* genes (commercial analysis is only available for mutations in these 2 genes). Individuals with MSI-low or MSS tumors are unlikely to harbor germline mismatch repair gene mutations, and further genetic work-up can be suspended. In families in which tumor tissue is not available, consideration can be given to

germline testing. In those families or individuals that meet any of the first 3 of the Bethesda criteria modified, germline testing without initial MSI analysis or in the presence of microsatellite stability can be considered. Recently, MSS has been found in the tumors of patients with *bMSH6* germline mutations,^{131–133} but the clinical implications are not resolved and remain an area of research.

Mismatch repair gene testing. Mismatch repair (MMR) gene testing is indicated for confirmational or presymptomatic testing in adults affected with or at risk for HNPCC (Table 9). This strategy for use of MMR gene testing has been espoused by a consensus panel¹⁰⁰ and expert opinion.^{128,129} Currently, commercial analysis is only available for mutations in *bMSH2* and *bMLH1*. Genetic testing is offered to individuals putatively affected with HNPCC whose tumor tissue has tested MSI-high (Figure 2 of the adjoining Medical Position Statement). Table 2 describes methods of testing for mutations of the mismatch repair genes. Although protein truncation testing is preferred for FAP, PTT has lower sensitivity for mutations in the mismatch repair genes. Therefore, sequencing, CSGE, or SSCP is favored for MMR gene testing. Future introduction of conversion technology may increase the sensitivity for detection of MMR gene mutations to 96%.^{31,130}

If a mutation is found in an affected family member, then genetic testing in at-risk relatives will provide true positive or negative results. Appropriate screening strategies can then be undertaken based on the at-risk person's gene test result. If a pedigree mutation is not identified, further testing of at-risk relatives is suspended because any negative gene test will be inconclusive. Such a result could be a false negative because the analysis is not capable of detecting a mutation even if present, or a disease-causing mutation may be present in other known MMR genes, or in yet undiscovered genes.

When an affected family member is not available for evaluation, starting the testing process with at-risk family members can provide only positive or inconclusive results. In this circumstance, a true negative test result in an at-risk individual can only be obtained if another at-risk family member tests positive for a mutation. This strategy is not preferred because of the high likelihood of inconclusive test results.

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Glossary of Test Methodology

Allele-specific oligonucleotide analysis (ASO): ASO analysis is a modification of Southern blotting. Small segments of DNA containing the mutated segment of the gene are amplified using PCR, dot blotted onto filters, and then hybridized with probes specific for known mutation sequences. ASO analysis detects known small deletions or single basepair substitutions that would be missed on routine Southern blot with a full-length cloned DNA probe. ASO screening only detects one specific mutation.

Conformational sensitive gel electrophoresis (CSGE) or heteroduplex analysis: CSGE has a sensitivity to detect mutations at greater than 90%. A drawback is that subsequent sequencing is needed to characterize mutations. To perform heteroduplex analysis, a segment of DNA containing the area of interest is amplified by PCR. In DNA from an individual with a mutant allele, CSGE amplifies the DNA from both the normal and the mutant allele. The amplified DNA is heat denatured and then allowed to reanneal at lower temperatures. If 1 allele has a mutation, the reannealed DNA will form heteroduplexes between the mutant and normal DNA and mutant-mutant and normal-normal homoduplexes. When run on a denaturing gel, the 3 different forms of double-stranded DNA show different mobility and result in 3 distinct bands. In individuals who carry 2 normal alleles, only 1 band is visible.

Conversion (previously known as monoallelic mutation analysis [MAMA]): Conversion uses the method of isolating a single allele in a rodent somatic cell hybrid. This can be considered a preparation step for mutation detection in that it enriches the analytic sample for only the chromosomes that bear the mutation of interest. Consequently, single alleles can be analyzed by mutation screening methods to detect mutations in heterozygotes that would be missed by conventional analysis.

Denaturing gradient gel electrophoresis (DGGE): DGGE is similar to SSCP. Mutant and normal DNA is denatured into single strands and allowed to reanneal. Fragments from a polymerase chain reaction are run on gels that contain an increasing gradient of urea and formaldehyde. These reannealed products have differing mobility on electrophoresis. Even single-base pair changes may alter the temperature or denaturant concentration at which the DNA strands uncoil or melt. A positive DGGE result indicates that a mutation exists but requires sequencing to characterize the mutation.

Detection of virtually all mutations-SSCP (DOVAM-S): DOVAM-S is a modification of the traditional SSCP methodology. SSCP is the most widely used method for mutation scanning. With SSCP, single-base sequence changes can be detected by altered electrophoretic migration of one or both single strands on a non-denaturing gel. With DOVAM-S, conditions such as gel matrix, running buffer, temperature, and additive are altered to increase the detection of mutations.

Immunohistochemistry (IHC): Immunohistochemistry is used to assay for the presence of *bMSH2* and *bMLH1* gene protein products in tumor tissue. The presence of both normal *bMSH2* and *bMLH1* gene products in tumor tissue indicates

that it is unlikely that an altered form of either of these genes is present in the germline. The absence of one of these products directs germline testing to the responsible gene.

Linkage analysis: Linkage analysis investigates whether a pattern of DNA markers near the gene of interest segregates in a family with disease. When markers are closely linked with the disorder, they can predict the inheritance of the disease susceptibility gene. Linkage analysis can be used for genetic testing if the chromosomal location of a disease gene is known but the gene has not been identified, or if specific mutations of known genes cannot yet be detected. This genetic analysis requires the study of DNA from multiple family members, both affected and unaffected with the disorder.

Microsatellite instability testing (MSI): Repetitive DNA sequences of 1–4 nucleotides called microsatellites are normally found in many locations throughout the genome. MSI describes the abnormal phenomenon in which tissues acquire variability in the number of nucleotides in the repeat sequences. In tumors occurring in HNPCC patients, an acquired increase or a decrease in the number of nucleotide repeat sequences can be noted. This test can be used to determine whether a colorectal cancer is likely to be sporadic or associated with HNPCC. In those patients whose tumors demonstrate MSI, mutation testing for the mismatch repair genes could be offered to identify the responsible germline mutation.

Protein truncation test: The protein truncation test is a useful functional assay for mutations that result in truncated (foreshortened) proteins that are encoded by the gene, and are, therefore, likely (but not certain) to be harmful. Protein truncating mutations include frameshift, nonsense, and splice site mutations. In some genes, such as *APC* gene associated with FAP, the majority of mutations are truncating. The most important limitation of a protein truncation test is that it cannot detect nontruncating mutations that can also be deleterious. In addition, the assay is only a screening test and does not characterize the specific mutation involved.

Sequencing: DNA sequencing is thought to be the most sensitive method for detecting and defining mutations. Sequencing may be used as the primary method for detecting mutations, but at present it is an intensive approach. Although the detection of mutations is the highest with sequencing, mutations in promoter regions and introns may be missed. Large deletions and insertions also will be missed. Finally, sequencing is incapable of distinguishing between harmful missense mutations and natural polymorphisms, resulting in variance of unknown significance.

Single-strand conformation polymorphism (SSCP): This method detects between 60% and 95% of mutations in short DNA strands. SSCP is a mutation screening test, and subsequent DNA sequencing is needed to characterize the mutation. This method works by chemically denaturing of DNA into single strands. The resulting single strands fold upon themselves, and the shape of single strands with mutations may differ from those strands without mutations. This difference can be detected by electrophoresis because mobility of mutant and normal strands will differ.