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# Genetic testing for colon cancer: Joint statement of the American College of Medical Genetics and American Society of Human Genetics

*Joint Test and Technology Transfer Committee Working Group*

Colorectal cancer (CRC) is the second leading cause of cancer death in the United States. Annually, approximately 130,200 individuals will be diagnosed and 56,300 will die from this disease.[1] In general, CRC evolves in an "adenoma to carcinoma" sequence during which a series of somatic alterations accumulate in the DNA of the tumor tissue. Since 1987, significant strides have been made in characterizing the genetic events that lead to colorectal cancer. This work has been based on detailed clinical and molecular genetic studies of colorectal tumors. Acquired genetic alterations seen in tumors include APC and MCC on chromosome 5q, KRAS on chromosome 12p, DCC on chromosome 18q, GTBP (hMSH6) on chromosome 2p, and p53 on chromosome 17p.[2] In addition, development of these genetic alterations may be accelerated by molecular instability or chromosomal instability.[3–4] About 75% of the time, molecular alterations are sporadic events, but the remaining instances arise in individuals with a family history of colon cancer.[5]

An important strategy in identifying genes involved in colorectal neoplasia has been the study of colorectal cancer syndromes, through which it has been found that specific genes are the basis of inherited cancer susceptibilities. Mutations in several genes have been associated with hereditary cancer: APC gene in familial adenomatous polyposis (FAP) and the DNA 'mismatch repair' genes, hMSH2, hMLH1, hPMS1, hPMS2, and hMSH6 (GTBP) in hereditary nonpolyposis colorectal cancer (HNPCC).[6–10] A novel locus has been reported on chromosome 15q in a multiplex CRC family, and the gene has been named CRAC (colorectal adenoma and carcinoma).[11] Although these currently known susceptibility genes account for < 10% of all colorectal cancers, there remains at least 20% of patients who have family histories of colon cancer and for which mutations in genes remain to be identified.[5,7]

### Familial adenomatous polyposis

Germline mutations (primarily nonsense, frameshift) of APC are associated with FAP, an autosomal dominant syndrome, which is clinically characterized by young onset (age 12–15 years), hundreds of adenomatous polyps in the colon, and increased risk for gastric polyps, duodenal cancer, thyroid cancer, and desmoid tumors.[9] An attenuated variety (AFAP) has fewer than 100 adenomas with proximal predominance and later age of onset (55 years). In general, AFAP is associated with APC mutations that occur near the 3′ and 5′ ends of the gene.[12–14] Missense mutations in APC have also been reported in non syndromic colorectal neoplasia families.[15,16]

A recently discovered missense mutation in APC, known as APC I1307K, is associated with an increased risk for colorectal adenomas and carcinoma, but not as high as in FAP.[17] This mutation, which has been found to occur only in the Ashkenazi Jewish population, with a prevalence of 6%, is found in 10% of colorectal cancer patients who are of Ashkenazi Jewish heritage, and up to 28% of such patients who also have a positive family history of colon cancer. This particular mutation does not in itself cause polyposis or cancer, but instead is a true cancer predisposition gene, because it creates an instability in the colon cell's APC gene (making it hypermutable) that then may develop a more deleterious mutation that can lead to cancer. This novel mechanism for cancer predisposition may partially explain the reduced penetrance of the mutation. Others have confirmed this observation[18–20]; for example, Woodage et al.[18] have confirmed the relatively high frequency of this mutation, finding 7.2% of over 5,000 Ashkenazi Jews to be carriers.

### Hereditary non-polyposis colon cancer

Germline mutations in five mismatch repair-related genes (hMSH2, hMLH1, hMSH6, hPMS1, and hPMS2) cause

*This guideline is designed primarily as an educational resource for medical geneticists and other health care providers to help them provide quality medical genetic services. Adherence to this guideline does not necessarily ensure a successful medical outcome. This guideline should not be considered inclusive of all proper procedures and tests or exclusive of other procedures and tests that are reasonably directed toward obtaining the same results. In determining the propriety of any specific procedure or test, the geneticist should apply his or her own professional judgment to the specific clinical circumstances presented by the individual patient or specimen. It may be prudent, however, to document in the patient's record the rationale for any significant deviation from this guideline.*

HNPCC, and are associated with specific somatic alterations in the tumor, characterized by high microsatellite instability (MSI-H).[21,22] HNPCC is characterized by young onset colorectal cancer (mean age 44 years), proximal colon location, multiple primary cancers, and increased risk of endometrial cancer, transitional cell cancer of the ureters, small bowel cancer, gastric cancer, bile duct cancer, and ovarian cancer.[23,24]

Microsatellite instability (MSI) in the tumor is associated with HNPCC and tumors with specific pathologic characteristics, suggesting a group of patients on whom such analysis may be useful.[21,22] Recently, in a large retrospective study, MSI has been found to be associated with improved survival in young colorectal cancer patients, at all stages, potentially heralding the use of MSI in treatment decisions or risk management.[25]

## Technology

*Germline genetic testing*

Gene tests for colorectal cancer (see Table 1) in the clinical setting primarily consist of the following:

- Protein truncation testing (in vitro synthesized protein assay) for truncating germline mutations of APC, MSH2, and MLH1 in patients where the mutation is not known. This method detects disease-causing mutations (nonsense or frameshift) that result in premature protein termination. In particular, APC mutations in the majority of patients with classic FAP phenotype may be detected this way.[26–30] This method is less sensitive for HNPCC, because other types of mutations contribute to the disease phenotype.[31] If the truncated protein is detected by this assay in the affected patient, then the defect can be similarly assayed in a focused evaluation of at-risk relatives.
- CSGE, SSCP, or other screening assay, followed by DNA sequencing for MSH2 and MLH1 in HNPCC families where the mutation is not known. These methods are more sensitive than protein truncation testing, but may still not detect all mutations.[31–33] Once a disease-associated germline mutation is detected in an affected patient, then ASO methods (described next) can be employed to evaluate at-risk relatives.
- Allele specific oligonucleotide (ASO) hybridization is appropriate when the specific disease-causing mutation is known, in cases such as APC I1307K,[17] or predictive testing in an FAP or HNPCC kindred where the causal mutation has been identified.[31,34]

*Tumor assays for genetic predisposition*

The availability of archival paraffin-preserved colorectal tissue (normal and tumor) enhances our ability to evaluate patients or families suspected of harboring HNPCC mutations. There are two approaches that are moving into clinical application:

- Microsatellite instability (MSI) is considered to be a feasible and informative option in evaluating a family suspected of HNPCC.[21,22,35] Patients with colorectal cancer

**Table 1**
Genetic tests for susceptibility to colon cancer

| Cancer type | Test name | Method (reference) | Estimated analytic sensitivity/specificity | Sample | Indication |
|---|---|---|---|---|---|
| HNPCC | MSI, IHC | Microsatellite instability analysis (22) and immunohistochemical staining (38–40) | 85%/85% | Paraffin block (tumor) | Affected individuals with colon or uterine cancer in families with ≥3 cases of colon or uterine cancer, or early-onset colon cancer; if tumor manifests MSI, germline mutation analysis should be considered |
| HNPCC | MSH2, MLH1 | DNA sequencing (i.e., 31) | 70%[a]/99% | Whole blood (14 mL lavender top tube) | Affected individuals in families with greater ≥3 cases of colon or uterine cancer; if prior MSI tumor assay done, probability of germline mutation is low if tumor was microsatellite stable |
| HNPCC | MSH2, MLH1 | Protein truncation (28–30) | 50%[a]/99% | Whole blood (7 mL lavender top tube) | Affected individuals in families with ≥3 cases of colon cancer tested first; unaffected at-risk relatives tested only if affected patient mutation detected |
| FAP | APC | Protein truncation (26–27) | 75%/99% | Whole blood (7 mL lavender top tube) | Affected individuals tested first; unaffected at-risk relatives tested only if affected patient mutation detected |
| Familial colon cancer | APC I1307K | ASO (17) | 99%/99% | Whole blood (7 mL lavender top tube) | Affected and unaffected individuals of Ashkenazi Jewish ethnicity with family history of colon cancer |

[a]At least three other genes account for the other 30% of cases.

whose tumors are found to manifest MSI should be considered for further germline mutation analysis, particularly if the family history and the value of risk assessment for family members is warranted.[9,36] If the MSI result is negative or equivocal, one should still rely on the strength of the family history to make screening recommendations,[33–37] since up to 15% of colorectal tumors from HNPCC patients will not display the phenotype. Likewise, an equal number (15%) of apparently sporadic tumors are MSI positive.

- Promising new research suggests that immunohistochemistry (IHC) for MLH1 and MSH2 expression is an inexpensive first screen of CRC tumors to evaluate whether MSI and/or germline testing is indicated. Lack of expression of either MSH2 or MLH1 by IHC in tumors is correlated with MSI in the tumor.[38–40] The majority of MSI observed in sporadic cases appear to be due to somatic hypermethylation of the MLH1 promoter.[41,42] It has also been observed that absent MSH2 IHC expression is associated with germline MSH2 mutation, and a minority of absent MLH1 IHC expression is associated with germline MLH1 mutation.[42] The translation of these new methods into routine clinical practice awaits large-scale validation studies.

*Future technology: conversion of diploidy to haploidy*

Recently, Vogelstein and coworkers have developed a new technique whereby mutations in APC, MSH2, or MLH1 may be detected by converting diploid cells to haploid cells by selectively fusing with mouse cells and screening the human chromosome of interest. Also termed monoallelic mutation analysis, this method may be more widely available in the near future.[32,43]

**Unresoved issues**

There are a number of issues that need to be considered in the context of colon cancer genetic testing options. The characterization of genetic susceptibility to colon cancer is a very active area of research, and until studies on large populations of patients are completed, precision in risk assessment and screening or preventive interventions will be based on current best evidence. With respect to FAP and HNPCC, genetic tests are part of the spectrum of clinical information gathering, through which management options can be developed. The clinical, technical, and psychosocial frameworks (both the medical as well as the patients' point of view) should be incorporated in testing strategies.

*1. How many genes are going to turn out to have an effect on colon cancer, especially the hereditary form?*

For example, the syndrome HNPCC is due to a class of genes that are involved in the DNA mismatch repair pathway, so that essentially the identical clinical diagnosis can be explained by any of five different genes. While a majority of mutations are found in just two genes (MSH2 and MLH1), if no mutation is found in these two, there are few other clinical testing options. Only a few research laboratories are studying mutations in the other three genes. Yan et al.[32] suggest that the majority of HNPCC families that manifest MSI tumors will be accounted for by MSH2 and MLH1. In total, however, mutations in these genes only account for approximately one half of the families that fit the Amsterdam criteria (particularly those that do not manifest MSI); thus, additional genes are likely to be involved that may have other underlying mechanisms of action.

*2. What is the clinical spectrum associated with mutations in "colon cancer" genes?*

Molecular diagnostic testing can better characterize syndromes or even split syndromes that were originally considered a single entity on clinical observation grounds. For example, there are more cancers of the renal pelvis, ureter, stomach, and ovaries when the family harbors a mutation in MSH2 (vs. MLH1), while mutations in MLH1 that completely silence the gene product (i.e., no protein produced) have a paucity of extracolonic tumors. An atypical phenotype is seen in families with MSH6 mutations where endometrial/ovarian cancers outnumber colorectal cancers. Further differentiation must await long-term follow-up of multiple families with well-characterized mutations.

In another example, Turcot syndrome was thought to be an autosomal recessive condition characterized by colonic polyposis and brain tumors. Mutation analyses have now shown that Turcot syndrome can be split into at least two dominant polyposis syndromes: (1) the true Turcot syndrome, which is associated with glioblastoma and is due to mutations at MLH1 and PMS2; and (2) the Crail syndrome, which is associated with a different brain tumor and medulloblastoma, and is due to mutations in APC.[44]

As geneticists study the action of these genes and the mutations in different regions, more subtle phenotypes are being associated with each [e.g., 15–16]. This finding has the potential to complicate the management of carriers until such time that the most complete spectrum of potential tumors has been identified. Individuals shown to harbor pathologic mutations should remain in an ongoing surveillance program.

*3. What is the best surveillance for carriers?*

The American Cancer Society, American Gastroenterology Association, and National Cancer Center Network have developed recommendations and practice guidelines that consider genetic risk for surveillance of individuals from familial or hereditary colorectal cancer pedigrees.[45,46] These recommendations are largely based on retrospective clinical observations and expert opinion, as there are no data from large-scale population-based or prospective studies of cancer risk in carriers.

Molecular testing has the potential to improve management of mutation carriers. For example, the attenuated form of FAP (AFAP), which is characterized by fewer adenomas and may be of later onset, should be diagnostically followed by colonoscopy rather than sigmoidoscopy as is standard for classic FAP.[47] HNPCC also requires colonoscopy to monitor for polyps.[48] It is the hope, anecdotally observed, that there would be

concomitant improvement in adherence to cancer prevention recommendations.

### Informed consent

The American Society of Clinical Oncology[49] has developed a list of elements of informed consent for cancer genetic testing, including (1) information on the specific test being performed, (2) implications of a positive and negative results, (3) possibility that the test will not be informative, (4) options for risk estimation without genetic testing, (5) risk of passing a mutation to children, (6) technical accuracy of the test, (7) fees involved in testing and counseling, (8) risks of psychologic distress, (9) risks of insurer or employment discrimination, (10) confidentiality issues, and (11) options and limitations of medical surveillance and screening following testing. It is recommended that all testing be done with a comprehensive informed consent document.

### Summary

Colorectal cancer gene discoveries have led to clinical application in the form of improved cancer genetic risk assessment and genetic testing for hereditary colon cancer, including FAP and HNPCC. However, much more continues to be learned about the biology and clinical aspects of colon cancer susceptibility.

- Germline gene test for FAP utilizes a protein truncation test of APC.
- Germline gene test for APC I1307K is an ASO-based assay and may identify persons of Ashkenazi Jewish origin who are at increased risk for colon neoplasia.
- HNPCC is genetically heterogeneous but is largely due to mutations in MSH2 and MLH1. Mutations in these mismatch repair genes often result in microsatellite instability (MSI) in the tumor. Tumor assays for MSI may help identify persons who are likely to carry germline MSH2 or MLH1 mutations.
- Germline gene tests for MSH2 and MLH1 utilize protein truncation and DNA sequencing strategies.
- Genetic counseling is an important and crucial component of the genetic risk assessment process. Informed consent for genetic testing is an integral part of the process, and a clear understanding by the patient can only be arrived at by careful counseling.
- In view of the complicated and evolving technology and clinical issues, individuals with a family history of colon cancer would be best served in programs with appropriate laboratory and clinical expertise.

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*9650 Rockville Pike, Bethesda, MD 20814-3998*
*Phone 301-530-7127, Fax 301-571-1895*

***Joint Test and Technology Transfer Committee Working Group***
*Patricia Murphy, PhD, Co-Chair*
*Gloria Petersen, PhD, Co-Chair*
*Stephen Thibodeau, PhD*
*Richard Fishel, PhD*

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