DNA TESTING STRATEGIES AIMED AT PREVENTING HEREDITARY NON-POLYPOSIS COLON CANCER (HNPCC)

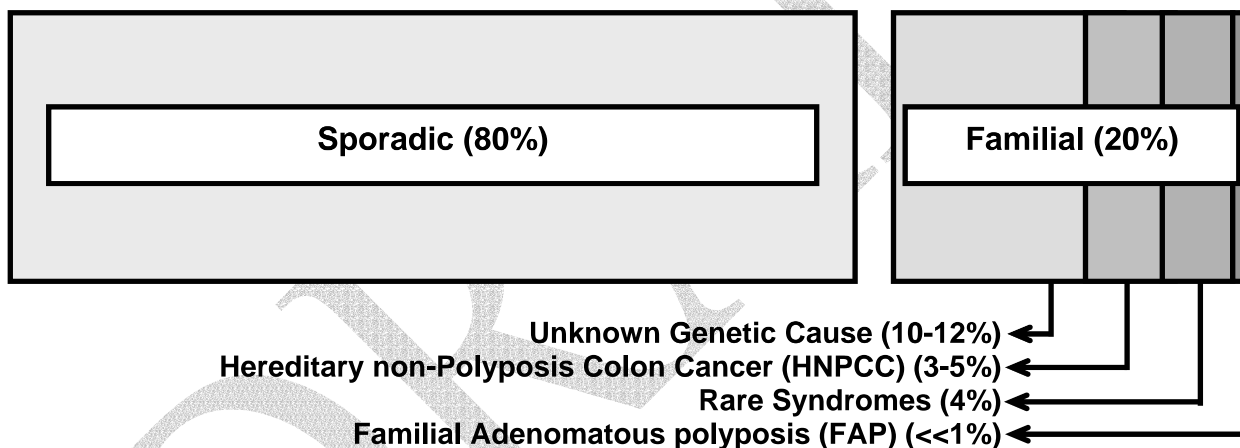
DISORDER/SETTING

1. What is the specific clinical disorder to be studied?

The main clinical disorder being studied in this review is a specific form of inherited colorectal cancer, termed hereditary non-polyposis colorectal cancer (HNPCC). In the United States in 2003, there were 147,500 new diagnoses and 57,100 deaths involving colorectal cancer. Colorectal cancer is the third most common cancer type and the third most common cause of cancer death in both men and women (American Cancer Society, 2003). Figure 1 shows that about 200 of every 1,000 diagnosed colorectal cancer cases will be familial, meaning that a relative will also have colorectal cancer (Trimbath and Giardiello, 2002). Of these 200 familial cases, about 40 will be explained by HNPCC. This is the most common Mendelian form of colorectal cancer.

The genetic basis for the remainder of inherited colorectal cancer cases (other than familial adenomatous polyposis) is largely unknown.

Figure 1. Epidemiology of Colorectal Cancer



2. What are the clinical findings defining this disorder?

An inherited susceptibility to colorectal cancer is usually first suspected because of multiple affected individuals in a family, an unusually young age at diagnosis, or multiple colorectal cancers in one individual. Alternatively, it may be suspected because of certain extra-intestinal manifestations. Hereditary nonpolyposis colorectal cancer (HNPCC), also called Lynch syndrome (Lynch *et al*., 1966), is an autosomal dominant disorder, accounting for about 3 to 5 percent (range, 1 to 13 percent) of all colorectal cancers (Mecklin, 1987; Scapoli *et al*., 1994; Rodriguez-Bigas *et al*., 1997; Aaltonen *et al*., 1998). A person with HNPCC may have multiple primary tumors. The known causes are mutations in genes governing mismatch repair. Individuals with HNPCC have a 70 to 80 percent lifetime risk of developing colorectal cancer (Scapoli *et al*., 1994; Vasen *et al*., 1996). Colorectal cancer develops at an average age of 44 years (Lynch *et al*., 1993). Tumors arise most often on the right side of the colon in HNPCC (60 to 80 percent); in sporadic colorectal cancer, most tumors arise on the left side (68 to 77 percent). Precursor adenomas are frequently villous. Carcinomas tend to be poorly differentiated with abundant mucin and lymphoid infiltration. Survival is better stage-for-stage than with sporadic colorectal cancer (Gryfe *et al*., 2000). Men with the genetic predisposition develop cancer more of-

ten (80 percent) than women (40 percent) during their lifetime (Mitchell *et al.*, 2002). However, women with HNPCC are also at risk for malignancies involving the endometrium (39 to 60 percent) and ovary (9 percent) by age 70 (Cruz-Correa and Giardiello, 2002) (Table 1). For both men and women, there is an increased relative risk of transitional cell carcinoma of the ureter and renal pelvis and adenocarcinoma of the stomach, small bowel, and biliary system, ranging from 3 to 25 times the risk in the general population (Vasen *et al.*, 1990; Watson and Lynch, 1993; Aarnio *et al.*, 1995).

Table 1. Extracolonic Disorders Associated with Hereditary Nonpolyposis Colorectal Cancer[a] (HNPCC)

Cancers	Other Lesions
Brain (glioblastoma)[b]	Café au lait spots
Stomach	Sebaceous gland adenomas & carcinomas[c]
Small bowel	Keratoacanthomas[c]
Biliary tract	
Ureter and renal pelvis	
Uterus	
Ovary	

[a] From Giardiello *et al.*, 2001
[b] Turcot's syndrome is characterized by glioblastoma associated with HNPCC
[c] Muir-Torre syndrome is a variant of HNPCC characterized by sebaceous gland tumors and/or keratoacanthomas.

3. What is the clinical setting in which testing is to be performed?

This colorectal cancer ACCE Review has as its main aim to evaluate the efficacy of identifying hereditary non-polyposis colorectal cancer (HNPCC) mutations among individuals with newly diagnosed colorectal cancer and then testing for the mutation in their first degree relatives, for the purpose of offering preventive measures. At present, testing is readily available for identifying mutations in two selected genes (*MLH1* and *MSH2*), that are responsible for up to 90 percent of HNPCC cases. The high cost of such testing and the relatively low frequency of HNPCC mutations among individuals with newly diagnosed colorectal cancer precludes its use in all such cases. Instead, preliminary evaluations including family history of colorectal cancer and microsatellite instability (MSI) testing (see Question 6) have been developed to identify cases in which mutation testing might be warranted. In 1990, the Amsterdam criteria for diagnosis of HNPCC were proposed to identify index cases for research purposes (Vasen *et al.*, 1991). To take account of cancers in extracolonic sites, the Amsterdam criteria II were later proposed. The Amsterdam criteria have been criticized as too restrictive for clinical use. Hence, new criteria, called the Bethesda criteria (Table 2), have been developed for selecting patients whose tumors are suitable for MSI testing (Umar *et al.,* 2004). The Bethesda criteria are more sensitive, but less specific, than the Amsterdam criteria.

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Table 2. Bethesda Criteria for Testing Colorectal Tumors for Microsatellite Instability[a] (Testing is indicated using any one of the following criteria)

Individuals with cancer in families that meet the Amsterdam criteria

Individuals with 2 HNPCC-related cancers, including synchronous and metachronous colorectal cancer or associated extracolonic cancers[b]

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,[c] and the adenoma diagnosed at age <40 years

Individuals with colorectal cancer or endometrial cancer diagnosed at age <45 years[c]

Individuals with right-sided colorectal cancer with an undifferentiated pattern (solid/cribriform) on histology diagnosed at age <45 years [c, d]

Individuals with signet-ring cell-type colorectal cancer[e] diagnosed at age <45 years

Individuals with adenomas diagnosed at age <40 years

[a] From Giardiello *et al*., 2001.
[b] Endometrial, ovarian, gastric, hepatobiliary, small bowel, or transitional cell carcinoma of the renal pelvis or ureter.
[c] Guidelines for age of cancer diagnosis have been adapted to <50 years in the American Gastroenterological Association (AGA) Medical Position Statement (Bethesda criteria modified).
[d] Solid/cribriform defined as poorly differentiated or undifferentiated carcinoma composed of irregular, solid sheets of large eosinophilic cells and containing small gland-like spaces.
[e] Composed of >50 percent signet ring cells.

The American Gastroenterological Association (AGA) recommends genetic testing for affected individuals in families meeting the Amsterdam or Bethesda criteria and first-degree adult relatives of those with known HNPCC mutations (Giardiello *et al*., 2001). If a deleterious mutation is found in an affected family member, genetic testing of relatives can provide definitive results. If no deleterious mutation is found in the affected person (or if an affected family member is not available for evaluation) testing an at-risk family member can provide a positive, but not a definitively negative, result. Web sites listing current recommendations for the AGA can be found at www.gastro.org and for the PDQ Cancer Genetics Editorial Board at www.cancer.gov/cancerinfo/pdq/genetics.

Gap in Knowledge The proportion of individuals that has a positive family history by the Bethesda Criteria, but are found not to have HNPCC, has not been reported.

4. What DNA test(s) are associated with this disorder?

Preliminary family history questions. Asking questions about family history using the Amsterdam or Bethesda criteria represents the first level of preliminary evaluation of individuals with

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newly diagnosed colorectal cancer to determine whether further testing is warranted. The Bethesda criteria (Table 2) identify what clinical information is needed to assess risk, including who in the family has had what kind of cancer, at what age, how they are related to the index patient, whether the patient's colon cancer is right- or left-sided, its histology, and whether the patient has had adenomas. All individuals satisfying the Amsterdam criteria will automatically satisfy the Bethesda criteria.

Preliminary laboratory testing. When an index case satisfies the Bethesda criteria, the next step in the process involves testing the tumor for MSI. MSI is found in the DNA of colorectal cancers of individuals with mismatch repair gene mutations, but not in the DNA of adjacent normal colorectal mucosa or in DNA from other normal tissues of the same individual. Microsatellites are DNA sequences, usually consisting of 1 to 5 nucleotide repeats. For example, the most common human microsatellite is a dinucleotide repeat of C and A, $(CA)_{11}$. MSI refers to a gain or loss in the number of repeats in DNA in a tumor, compared to the number of repeats in the same region in non-tumor DNA from the same individual. MSI is recognized by observing multiple bands on electrophoresis of PCR products using tumor DNA where fewer bands are seen using PCR products of non-tumor DNA. The multiplicity of bands results from the failure to correct transcription errors. Such "infidelity" exposes the important role of the gene products of the mismatch repair genes in recognizing and correcting errors in DNA transcription. Errors are most likely in regions of short repeated sequences (microsatellites) which may induce slippage of DNA polymerase, resulting in insertion or deletion of the repeated units. This instability is also referred to as the "mutator phenotype" or "replication error phenotype (REP)". For the MSI test to have a negligible false negative rate, the sample must have a high proportion of tumor cells. To ensure this high proportion, a pathologist is needed to separate tumor cells from non-tumor cells on tumor sections. MSI testing is the most commonly used preliminary laboratory test, and emphasis will be placed on this test rather than the alternative immunohistochemical test discussed below.

Some laboratories use an alternative immunohistochemical (IHC) technique to detect mismatch repair gene products. IHC can be done on tumor samples to determine whether *MLH1* or MSH2 gene products are present. It constitutes an alternative method to MSI testing for selecting patients likely to have a detectable mutation. Advantages of IHC include low cost and an indication as to which gene to sequence first. Disadvantages are the fact that experience is needed in evaluation of the fluorescent pattern obtained and that a lack of *MLH1* protein is often due to somatic hypermethylation of the MLH1 promoter, rather than to an alteration in the DNA sequence (i.e., false positive results).

DNA Testing

When DNA sequencing was done manually, it was common (among index cases with positive MSI test results) to employ a simpler procedure first and to completely sequence only those samples found to be abnormal. These simpler procedures included single strand conformation polymorphism analysis, denaturing gradient gel electrophoresis, Southern blotting, and more recently DHPLC. The most sensitive of these DNA-based procedures is DHPLC, the only method detecting 100 percent of mutations in a comparison of four methods (Eng *et al.*, 2001a). However, most clinical laboratories now use automated DNA sequencing, and one laboratory (Myriad Genetic Laboratories) has also automated the large number of polymerase chain reactions required. Thus, the less sensitive methods have given way to the highly sensitive DHPLC as the method of choice and, with greater use of automated sequencing, there is a trend to move directly to sequencing.

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DNA sequencing cannot be performed on tumor tissue (see Question 15, for further explanation). Once MSI has been identified in tumor tissue in an index case, the next recommended step is to obtain a sample (usually blood) for DNA sequencing. The known causes of HNPCC are mutations in one of several DNA mismatch repair genes (Table 3). These genes normally maintain fidelity of DNA replication by correction of nucleotide mispairs and small insertions or deletions generated by misincorporation or slippage of DNA polymerase during transcription. Sequencing is performed customarily only for *MLH1* and *MSH2*, because these two genes account for about 90 percent of the mutations detectable via sequencing (Cruz-Correa and Giardiello, 2002; Lynch and de la Chapelle, 2003). The commercially available DNA tests for identifying mutations associated with HNPCC are listed in Table 4 (Giardiello *et al.*, 2001). A review of uncommon mutations causing HNPCC is maintained at www.nfdht.nl\database\mdbchoice.htm.

Table 3. Mismatch Repair Genes: Site of Mutation in Hereditary Non-Polyposis Colon Cancer (HNPCC) and Proportion of HNPCC Mutations Detected Using Conventional DNA Sequencing

Gene	Chromosome Location	Proportion of all HNPCC Mutations Associated with Individual Genes (%)*
MLH1	3p21	50.9
MSH2	2p16	37.6
MSH6	2p16	9.6
PMS1	2q31	0.3
PMS2	7q11	1.6

*Only a proportion of these mutations is detectable using tests available for clinical purposes

HNPCC Mutations Not Detected by Conventional DNA sequencing

Conventional DNA sequencing described above cannot detect all disease-causing mutations in the HNPCC genes listed in Table 3. Large deletions are missed and may constitute 20 to 25 percent of all disease-causing mutations (de la Chapelle, 2003a; Wagner 2004). RNA-based methods can be used to find large deletions, if DNA-based detection methods reveal no abnormality (Wahlberg *et al.*, 1999). RNA-based methods involve synthesizing and sequencing complementary DNA (cDNA), representing the whole coding region. A mutation involving a deletion spanning an intron-exon junction is detected as a shortened fragment. However, major disadvantages are that defective alleles may be only weakly expressed or not expressed, and alternative transcripts may confuse the analysis (Kohonen-Corish *et al.*, 1996).

In sequencing, the normal allele often obscures the mutant allele. “Conversion technology” provides haploid cells for DNA analysis by fusing the patient’s lymphocytes to an established mouse fibroblast line and, through the serial spontaneous loss of human chromosomes, isolating cells that are haploid for the chromosome of interest. In one study of 22 HNPCC MSI-positive patients, conventional sequencing of diploid cell DNA identified a mutation in only 12 patients, whereas sequencing of hybrid haploid cells revealed a mutation in all 22 (Yan *et al.*, 2000).

Multiplex probe ligation tag assay (MLTA) is an automated procedure that can identify some of the mutations that are not identified by more standard methodologies. These more elaborate tests are suitable for research purposes, but are not practical for use in clinical practice.

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Table 4. Commercially Available Genetic Tests for Hereditary Non-Polyposis Colon Cancer (HNPCC)[a]

Analysis	Method	Cost if mutation is Unknown	Cost if mutation is Known
MSH2, MLH1	Sequencing	$800-$3000	$200-$500
MSH2, MLH1	CSGE[b] + sequencing	$1540	$260
MSH2, MLH1	SSCP[c]	$800	$250
MSH2, MLH1	Protein truncation	$750	$500
MSI testing of tumor	Polyacrylamide gel Electrophoresis	$300 to $500	

[a] From Giardiello et al., 2001.
[b] CSGE, conformation strand gel electrophoresis
[c] SSCP, single-strand conformation polymorphism
See www.genetests.org for details concerning specific laboratories

Once a pathogenic mutation is detected in a family member with a relevant cancer, relatives without cancer can be offered testing with the expectation of a definitive result. Such testing, which has been called single-site testing, is simpler and less expensive ($200 to $500) than the comprehensive testing of the index family member.

5. Are preliminary screening questions employed?

One study tested a cohort of colorectal cancers in Ohio and Finland using MSI testing, followed by DNA sequencing for those with positive MSI results (de la Chapelle, 2001, Altonen, 1998). In that study, 86 percent of persons found to have an HNPCC mutation were documented to have a positive family history. Thus, 14 percent of individuals (one in seven) who have colorectal cancer and an HNPCC mutation will have a negative family history. In practice, a policy that included MSI testing for all colorectal cancer cases would have a low yield and a relatively high cost per HNPCC patient identified. For that reason, preliminary questions regarding family history (e.g., the Bethesda criteria – Questions 3 and 4) of colorectal cancer (and associated cancers) are useful to identify a subset of patients for MSI testing.

Since the family history is so important for identifying high-risk individuals, the question arises as to its reliability. Many patients lack accurate knowledge of their family history of cancer. A Danish study compared the family history reported by consecutive colorectal cancer patients with that obtained from the Danish Cancer Registry. In 39 percent of cases, the proband lacked the family history information necessary to determine whether the family met the Amsterdam criteria (Katballe *et al*., 2001). Another study compared patient statements about their relatives with the death certificates, pathology reports, and the self-reports of relatives. The patients reported 89 percent of colon cancers in first-degree relatives and only 57 percent of colorectal cancers in second-degree relatives (Anton-Culver, 2003).

6. Is it a stand-alone test or is it one of a series of tests?

7. If it is part of a series of tests, are all tests performed in all instances (parallel) or are some tests performed only on the basis of other results (series)?

Taking a family history and performing microsatellite instability analysis of tumor cells are both considered here to be preliminary tests. The term “susceptibility” is used to acknowledge the

fact that some family members with an HNPCC mutation identified as a consequence of finding a mutation in an affected relative will not develop colon cancer within their lifetime. An inherited susceptibility to colorectal cancer is generally determined as the result of a series of tests. In a colorectal cancer patient with no or few polyps, HNPCC is the first consideration, and MSI testing is the usual next step among index cases satisfying the Bethesda criteria. If the MSI test is abnormal, but no mutation is found on DNA sequencing, some laboratories perform special procedures, such as Southern blotting or RNA analysis, to look for large deletions or large insertions. Laboratories differ as to the protocol followed. Some laboratories do DNA sequencing directly, while others perform another laboratory test and sequence only if that other test reveals an abnormality. Sequencing per se is generally not an option on a laboratory's testing requisition.

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